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AUTHOR'S OMISSION

The Use of Tetrachlorethane for Commercial Glasshouse Fumigation

BY THEODORE PARKER

Owing to an oversight the following references were unfortunately omitted from this paper:

"*Red Spider. A Note on its Control*"
T.P. Bulletin No. 5. March 1922. Bureau of Biotechnology, Murphy & Son, Ltd. pp. 143–149

"*The Fumigation of Commercial Glasshouses*"
T.P. Bulletin No. 9, vol. II. March 1923. Bureau of Biotechnology, Murphy & Son, Ltd. pp. 21–31

THE TRANSMISSION OF POTATO MOSAIC TO TOMATO

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(With Plates XXIV–XXVI.)

INTRODUCTION.

TRANSFERENCE of virus disease in potato to other potatoes or other hosts is complicated by two facts which were unknown to, or imperfectly appreciated by, the earlier workers, and invalidate some of their conclusions. The first of these is the existence of carriers. A given variety of potato may show no sign of disease, growing healthily and giving a good yield, and yet it may be carrying in a masked condition a virus disease, *e.g.* streak, which will produce the most marked symptoms in another variety on transference to it. Further, a particular potato may be obviously infected with one disease, *e.g.* mosaic, and at the same time be carrying a second disease of which it shows no symptoms, but on inoculation to another variety, intolerant of this second disease, the new host may develop the signs of the second disease, sometimes in the most unexpected form, and show little or no sign of the first disease which was obvious in the original host. As Atanasoff(1) says, one of the common difficulties in potato virus work is the appearance of an entirely different disease in the artificially infected plants. Unless it has been shown by careful preliminary tests that concealed disease is not present, the results of inoculation into another host may be most misleading.

The second complication is the possibility, suggested by Johnson(2), that material from apparently perfectly normal potatoes may have the property on inoculation into tobacco of evoking a virus disease in the new host. Whether this is simply a special case of the carrier will be referred to later on, but many of the aberrant results recorded in the literature are certainly to be attributed to this phenomenon.

Attempts to transmit potato virus disease to other hosts have been reported by several observers. Quanjer(4) failed to transmit potato "mosaic" to tobacco by grafting, and Schultz and Folsom(5) also failed

to transmit potato mosaic to tobacco either by leaf-mutilation or by spinach-aphids. There seems, indeed, to be no definite record of successful transmission of any potato virus disease to tobacco, before the experiments of K. M. Smith(6), who produced ring-spot by leaf-mutilation inoculation of material from Arran Victory potatoes affected with mosaic. Transference to tomato, however, has been recorded in a number of cases. Quanjer(4) transmitted potato "mosaic" by grafting, though not with all varieties of potato, and he states that crinkle, aucuba and leaf-roll are all transmissible to tomato, though in leaf-roll no signs may appear in the tomato and regrafting back to healthy potato may be necessary to demonstrate that transmission has occurred. Schultz and Folsom(5) by leaf-mutilation also succeeded in transmitting mild mosaic and rugose mosaic to tomato, getting signs in the new host similar to those in the original potato. Inoculation with streak material also produced disease in tomato, though this disease might be more like rugose mosaic than streak. Vanterpool(7), Fernow(8), Berkeley(9) and others record the development of a peculiar mottling in tomato on inoculation with potato virus material of different kinds, the appearance in the tomato usually bearing no resemblance to that in the potato and sometimes developing even when the inoculum was derived from potatoes showing no signs of disease. Some at least of these results may be attributed to the phenomenon described by Johnson who indeed says(3) that "the host range of potato viruses is apparently restricted to the potato." Even K. M. Smith's transmission of mosaic to tobacco might be included in this group, were it not for his failure to obtain any such disease with material from potatoes of the same variety proved to be free from virus.

The experiments described in this paper deal chiefly with the transmission of mild mosaic of potato to tomato and with the characters of the disease so produced. No disease was obtained in tomato on inoculating with material from healthy potatoes, but a definite and characteristic disease was regularly produced on inoculation with material from potatoes infected with mild mosaic.

Methods.

The method of inoculation was the same in all cases. Leaves were taken from the potato, minced and thoroughly ground in a mortar, then 3 c.c. of distilled water for each 1 gm. of tissue were gradually added with renewed grinding, and the resulting liquid was inoculated to at least six, usually eight, tomatoes. The liquid was dropped on a leaflet supported on a wooden slip and the leaflet scratched with a needle

through the liquid. Forty to fifty scratches were made per leaflet, and four leaflets were inoculated per plant. This gave an inoculation which was perhaps unnecessarily heavy, but it was intended to ensure that in the inoculation with normal material a sufficient dose should be given. The tomato plants were always quite young, growing rapidly and with only three to four leaves showing leaflets large enough for inoculation. They were grown in insect-proof cages until used, and after inoculation were usually returned to the cages, though sometimes, owing to lack of available space, the inoculated plants were kept on the bench in the glass-house, which was fumigated regularly. All plants were grown at temperatures over 50° F. The variety of tomato chiefly used was Kondine Red; but other varieties, such as Blaby, were also used, without difference in the results.

Normal potatoes.

Leaves from normal potatoes of nine different varieties have been so inoculated, viz. Majestic, Arran Chief, Arran Victory, Epicure, Sharpe's Express, Great Scot, President, Abundance and King Edward. I am indebted to Dr Salaman, Dr G. F. Pethybridge and Mr H. Bryan for much of this material. The greatest care has been taken to ensure that these potatoes did not harbour concealed virus. In a number of cases the stock from which foliage was taken had been repeatedly grafted by Dr Salaman with potatoes very susceptible to streak and mosaic, such as President and Arran Victory, without producing any disease in them; and I believe that all were in fact free from virus disease. The inoculated plants were held for at least four weeks, in some cases for six weeks or more. In no case was any disease produced in the tomatoes. In several instances leaves were taken from the inoculated tomatoes and again inoculated into a second generation of six to eight young tomatoes without producing any symptoms in them. I have had no case where a potato, which had been shown to be normal on preliminary testing, produced disease in tomato.

It seems clear that in this country and with these varieties of potato, potato protoplasm as such does not produce virus disease in tomato, and it is reasonable to suppose that the same would hold good of other varieties as well. One example may be given of the possible value of inoculation into tomato as a guide to mosaic infection in potatoes. A number of potatoes of the variety Kerr's Pink, which were apparently normal, had been grown by Dr Salaman. They had not yet been tested by grafting, and presented some very slight discoloration of the foliage,

which aroused suspicion. With his consent leaves of these plants were taken and inoculated to tomatoes, in which they produced characteristic signs of disease within fourteen days; and later, the original Kerr's Pink plants, from which these leaves had been taken, developed definite symptoms of mosaic disease.

Mosaic potatoes.

On the other hand, leaves from mosaic potatoes have invariably produced in tomato a definite disease, and up to the present all the inoculated tomatoes have shown it. The following varieties of potatoes infected with mosaic have been used: Majestic, Arran Chief, Arran Victory, Up-to-Date and Kerr's Pink. All have exhibited the same general symptoms, of which a description is given later. Three of these varieties were used in the normal series, when they produced no disease in tomato, and it is reasonable to conclude that the symptoms produced in the tomatoes were due to the mosaic present in the potatoes. Normal plants of Up-to-Date and Kerr's Pink have not yet been procured. The Up-to-Date potatoes used presented certain features of interest. As is well known, the Up-to-Date variety is a persistent carrier of streak, of whose presence it shows no signs, but when grafted with a susceptible variety such as Arran Victory, the latter goes down with an extreme form of streak disease. Dr Kenneth Smith gave me some shoots of Up-to-Date which had no visible signs of mosaic at the time, but the plant was known to be infected with mosaic. He also gave me some shoots of Arran Victory which had been grafted with the same Up-to-Date plant, and showed the characteristic lesions of very virulent streak. Leaves from the Up-to-Date were inoculated to tomato and produced the usual disease, but leaves from the streaked Arran Victory inoculated at the same time to eight tomatoes of the same batch and kept in the same chamber of the glass-house produced no symptoms at all. No conclusion, of course, can be drawn from a single instance, but this result would suggest that streak may not be transmissible to tomato, at least not by this method of inoculation. It is noticeable that the mosaic present in the Up-to-Date and able to produce lesions in tomato from that variety was not present in the Arran Victory in such a form, or perhaps in such a quantity, as to produce any perceptible effect in the tomato, though presumably the mosaic must have had the chance of passing with the streak to the Arran Victory through the graft. Arran Victory is, as already mentioned, susceptible to mosaic and can transmit it to tomato in the absence of streak.

Other examples have been found where potatoes showing mosaic produced the characteristic lesions in tomato, but they are not detailed in the present paper, since opportunity has as yet been lacking to make certain that they contained no other virus disease than the visible mosaic.

Characters of the disease.

The disease as it appears in the tomato may take either of two distinct forms, which, however, may occur concurrently. As a rule, the first signs appear within fourteen days in the form of small necrotic spots. These come quite suddenly, often first in leaflets of the same leaf as an inoculated leaflet, but also often first on the leaf next above the inoculated leaves. They are usually isolated at first (Plate XXIV, fig. 1), but rapidly increase in number and may eventually coalesce to form larger necrotic areas. This condition may remain the only symptom for many days, but usually some mottling develops, either on leaflets already spotted or on other leaves. The second form in which the disease may appear is a mottle, and on the whole this is more common than the pure necrotic spot type. This also appears first on leaflets near the inoculated leaflets or on the leaf next above the highest inoculated leaf, usually first near the tip of the leaflet, and spreads upwards in the plant as it grows. The type of mottle is a spotting of paler green, sometimes rather fine in grain, sometimes coarser (Plate XXIV, fig. 2) and the spots tend to coalesce, so that the whole area of leaf affected becomes irregularly chlorotic with spots of still paler colour visible in it. The spots may show a tendency to form small rings of pale green or yellow enclosing a darker centre, but this ring formation is not so well marked in tomato as in some other hosts. In many cases this mottling is the only obvious symptom throughout the life of the plant, no necrosis developing at any time, but in most of these plants a few necrotic spots develop, one or two in every leaf, similar to those seen in the first type. In the first transfer from potato there is a distinct tendency for the mottling to fade and become much less obvious after a time, and a plant which had well-marked signs three weeks after inoculation may show very little a month later. After several transfers in tomato this fading rarely occurs.

There is no necrotic streaking of the stems or petioles at any time. In this it differs definitely from the streak or stripe of tomatoes common in glass-houses, to which disease the necrotic spot type bears a considerable resemblance. The distribution of the signs is also unlike that of tomato streak. In the disease here described the uppermost younger leaves usually remain free from signs throughout. Even after many generations

in tomato, the upper part of the plant seems quite normal, and the plant looks as if it were growing out of the disease. This, however, is not the case: as the young leaves in turn grow older and larger, they also develop the mottle or spots, and the symptoms spread gradually up the plant. In streak, on the other hand, it is common to find the youngest leaves streaked and spotted even at the very tip of the plant, and in the mottle form of that disease the mottling occurs also on the youngest leaves. The mottle in streak, moreover, is of a different type, larger and more blotched, and more like ordinary tomato mosaic.

Potato mosaic is not very virulent for tomato. The infected plants, though their growth is less than that of control plants, develop well and produce flowers and fruit. When combined with the yellow or aucuba mosaic of tomato, however, it causes a very severe disease, which has all the characters of true streak (Henderson Smith (10) and cf. Vanterpool (7), Dickson (11)). Even after years of propagation in tomato without return to the potato, it regularly produces this virulent disease when associated with the yellow mosaic, whether the two are inoculated simultaneously, or either is added to a plant already infected with the other. When the juice of a plant so streaked is treated with 90 per cent. alcohol for one hour, the potato mosaic factor of the combination is inactivated (see infra, p. 525) and the treated juice gives a pure yellow mosaic infection.

As already stated, up to the present time every tomato inoculated directly from mosaic potato has shown signs of infection; and in the very numerous subsequent transfers made from tomato to tomato, whether with filtered or unfiltered juice, failures to obtain transmission in all inoculated plants have been rare, provided the plants are growing well. The effects in the tomato, however, are not uniform. Besides the fact that some plants may show mottling almost exclusively and some only spot-necrosis, the signs vary considerably in intensity in different plants. In some cases they may be so slight that they might easily be missed, in others they are strikingly obvious. This variability makes it difficult to determine whether there is any difference in the several strains of potato mosaic we have used, or whether the variety of potato in which the mosaic is found affects its virulence for tomato. With Arran Victory mosaic all the tomato plants inoculated developed pronounced spot-necrosis with scarcely any mottle (which, however, was well marked in later transfers from tomato to tomato), while with Up-to-Date mosaic the tomatoes all showed marked mottling at first and necrotic spots only later. There has been no difference in symptoms so constant or definite as to justify a distinction between the mosaics used. With Majestic mosaic the inoculation produced only a slight mottling in the first

generation of tomato, a very definite mottle in the second, and in the third conspicuous spot necrosis with much mottling. This apparent increase of virulence was maintained in transfers for over a year, but recent transfers have given very little spotting.

When brought back from tomato into normal potato again, the original disease is reproduced in the latter in a more intense form: a very obvious mosaic, which develops in two or three weeks. We have seen no necrosis of the leaves or stems of the inoculated potatoes. Even after long propagation in tomato, inoculation into potato still produces typical mosaic in the latter. Cuttings were taken from healthy plants of President and Arran Chief, grown in sand, and, later, in soil, and when of suitable size, were inoculated in the usual way with the mosaic originally obtained from Majestic potato and maintained in tomato for two years. Intense mosaic developed in all the inoculated plants in fifteen days, the controls remaining healthy.

From tomato the potato mosaic is readily transmissible by leaf inoculation to other solanaceous plants. Of fifteen different plants inoculated, two only, viz. *Solanum melogena esculentum* and *Physalis francheti*, developed no symptoms. In the others leaf symptoms appeared within three weeks in every case, viz. in *Datura stramonium*, *Nicotiana tabacum* (White Burley), *N. affinis*, *N. Sanderae*, *Solanum nigrum*, *S. dulcamara*, *S. villosum*, *S. nodiflorum*, *Hyoscyamus niger*, *Nicandra physaloides*, *Petunia violacea*, *Capsicum annuum*, *Salpingoglossis sinuata*. These symptoms are quite unlike those produced by the yellow mosaic, which is transmissible to all of these hosts except *S. dulcamara*, *Physalis francheti* and *Datura stramonium* (though in the last, inoculation in the stem produces a localised necrosis without leaf signs). The symptoms have a general resemblance to one another in most cases, except in *Nicandra physaloides* where they appear as rather large (about 4 mm.) yellowish spots or blotches on the upper leaves, and smaller black necrotic spots on the lower leaves which show also a general chlorotic yellowing. The resemblance is not very close, but there is a tendency to form small rings in many of the hosts. This is well marked in *Hyoscyamus niger* (Plate XXV, fig. 3) and *Datura stramonium* (Plate XXV, fig. 4). The rings are usually small, about 1 to 2 mm. in diameter, much smaller than those figured by K. M. Smith (6) as occurring in a different variety of tobacco, and probably smaller than the ring-spot described by Johnson (2). They appear as of a paler green with dark green centre, and tend to turn into spots, the centre also becoming chlorotic and occasionally even necrotic. In tobacco (Plate XXVI, fig. 5) the pattern is very like the spot-necrosis disease of Johnson, figured by Hoggan (12).

Characters of the virus.

The characters of the virus of potato mosaic outside the plant have been studied chiefly in juice prepared from infected tomatoes by methods fully described elsewhere (10). It is filtrable, and after filtration through first an L. 1 and then an L. 3 Pasteur Chamberland candle produces infection when diluted 1 in 1000 with distilled water (100 per cent. of inoculated plants) and 1 in 10,000 (40 per cent.), but not when diluted 1 in 100,000. The *p*H of the filtered juice varied in different samples from 5·9 to 6·4. After simple clarification of the crude extract by passage through one layer of filter-paper, the juice still failed to infect when diluted 1 in 100,000 but gave incomplete infection in 1 in 10,000.

The virus is less resistant to heat than either the yellow mosaic or ordinary tobacco mosaic, being inactivated in ten minutes at 80° C. in all cases, and sometimes at 70° C. (see Table I). In potato juice the action of heat is similar. Juice was extracted from President potatoes infected with the Majestic mosaic, and filtered through candles in the usual way. After heating for ten minutes at 50° C., this filtered juice produced 100 per cent. infection; at 60° C., 100 per cent.; at 70° C., 17 per cent.; at 80° C., no infection.

Table I.

Effect of Heat on the Virus of Potato Mosaic.

Temperature	Majestic virus (*a*)	Up-to-Date virus (*a*)	Arran Victory virus (*a*, *b*)	Arran Chief virus (*b*)	Kerr's Pink virus (*b*)
50° C.	100	100	100	100	83·3
60° C.	100	100	100	100	80
70° C.	20	0	0	50	0
80° C.	0	0	0	0	0
90° C.	0	0	—	0	0
Unheated	100	100	100	100	100

The figures denote the percentage of tomato plants which showed infection; six to eight plants were inoculated in every case.

(*a*) Test made with juice filtered through candles.

(*b*) Test made with juice passed through one layer only of filter-paper.

To alcohol the virus is also less resistant than the yellow mosaic, being inactivated by 90 per cent. after one hour's exposure, and sometimes by 80 per cent. (Table II). The filtered potato juice (Majestic mosaic in President), after one hour's exposure to 50 per cent. alcohol, produced 100 per cent. infection; to 60 per cent., 43 per cent.; to 70 per

cent., 29 per cent.; to 80 per cent., 57 per cent.; to 90 per cent., no infection. Here again the Majestic and Arran Chief strains showed higher resistance than Up-to-Date.

Table II.

Effect of Alcohol on the Virus of Potato Mosaic.

Alcohol %	Majestic virus (*b*)	Up-to-Date virus (*a*)	Arran Chief virus (*b*)
50	—	100	—
60	100	85·7	100
70	100	42·8	100
80	37·5	0	50
90	0	0	0
Untreated	100	100	100

For explanation of figures and letters, see Table I.

A similar difference was found in regard to ageing. In filtered juice (tomato) Majestic mosaic remains infective for five and a half months, the longest period yet tested. Up-to-Date mosaic, however, was found to be inactive after 12 weeks, the filtered juice having been kept in dull light in paraffin-stoppered tubes.

The virus in filtered juice withstood 20 per cent. chloroform for four hours at 27° C., and the dyes Auramine O and Meldola Blue, diluted 1 in 2000, for two hours at 27°. Acriflavin, 1 in 1000 for the same time at 27° C., did not wholly destroy it, and 1 in 5000 did not affect it perceptibly. Meldola Blue, 1 in 500, did not completely inactivate it. Formalin, 1 in 500 for two hours at 27°, apparently killed it, but 1 in 1500 did not reduce its infectivity.

Discussion.

The marked difference in appearance of the two types of symptoms, the spot-necrosis and the mottle, suggests the possibility that these potato mosaics are made up of a mixture of two viruses. This may be the case, but we incline to the view that there is only one virus present and the two types of symptoms indicate a difference in reaction of individual plants. In the alcohol and heat series no differentiation occurred—*e.g.* after treatment with 60 per cent. alcohol the juice might give a pure spot-necrosis reaction in one plant and a pure mottle in another plant of the same batch, inoculated at the same time with the same material and kept under the same conditions. The type of symptom is affected to some extent by external conditions—*e.g.* growing the

inoculated plants at temperatures over 70° F. markedly reduced the necrotic spotting and favoured the mottling.

The disease here described bears a very close resemblance to the spot-necrosis disease described by Johnson (2) as obtained in tobacco by inoculation with the foliage of normal potatoes. The character of the symptoms, the occurrence of both the mottle and the spot-necrosis types, the distribution of symptoms and the tendency of the plant to grow out of the disease, the apparent increase of virulence in some cases (not in all) on continued transference in the new host, the thermal death-point, are all so closely alike in the two diseases that it is difficult not to believe that they are very closely related, if not identical. We have, however, never produced this disease with the foliage of normal potatoes (cf. K. M. Smith) and always produced it with the foliage of mosaic potatoes. Whether the two diseases are the same or not, it is evident that the normal potato in the United States differs from the normal potato in this country: unless, indeed, the potatoes accepted by Johnson as normal were not, in spite of the precautions he took, free from a form of suppressed mosaic. It is doubtful whether it is possible by inspection alone, however careful and regular and long-continued, to determine whether a potato is or is not infected with mosaic.

There are certain points in which the disease here described differs from the spot-necrosis of Johnson. His virus is inactivated by one hour's exposure to 50 per cent. alcohol: ours is not inactivated by 70 per cent. for the same time and not in every case by 80 per cent. His virus is inactivated by simple keeping or ageing in most cases in less than twenty days and sometimes in ten days: the virus (Majestic) here described remained infective for more than five months in tomato juice. His virus therefore differs from those described in this paper in its less resistance to ageing and to alcohol. Similar differences occur among the viruses we have worked with. These fall into two groups, the one represented by Majestic mosaic, not wholly inactivated at 70° C. nor by 80 per cent. alcohol and remaining active in filtered juice kept for five months, the other by Up-to-Date mosaic, inactivated at 70° C. and by 80 per cent. alcohol and by simple ageing for 12 weeks (possibly sooner). There would seem to be a series of viruses, all producing similar symptoms and all closely related to one another but differing in susceptibility. At the one extreme we have the virus of Johnson, inactivated by 50 per cent. alcohol and by simple keeping for two or three weeks; at the other extreme the Majestic virus, much more resistant to both alcohol and ageing, while the Up-to-Date virus has an intermediate position.

Further work is necessary before we can conclude with certainty that these differences in susceptibility indicate a real and constant difference in the viruses themselves; but they undoubtedly suggest that there exist strains or varieties of the virus of potato mosaic, and that mild mosaic in the potato may be due to one or more of several allied virus strains, as yet indistinguishable by the symptoms they produce.

I have pleasure in thanking Miss M. M. Browne for the care and skill with which she has grown the many plants required in these experiments.

Summary.

Inoculation by leaf-mutilation with the foliage of normal potatoes produced no disease in tomato. Nine varieties of potato were tested.

Similar inoculation with foliage of mosaic potatoes produced a characteristic disease in tomato. Five varieties of potato were used, of which three had been tested in the experiments with normal foliage.

The characters of the disease are described. It is transmissible back to potato again and to other solanaceous plants. The virus is filterable, is still infectious after high dilution of the extracted juice, and remains active on keeping for several months. It is less resistant to heat and alcohol than ordinary tobacco mosaic.

The disease resembles closely the spot-necrosis disease described by Johnson as obtained by inoculation of tobacco with foliage of normal potatoes, the chief difference being the greater resistance of the potato mosaics here described.

It is probable that there exist several strains, differing in resistance, of the virus causing mosaic in the potato.

References.

(1) Atanasoff, D. (1925). *Phytopath.* xv, 171.
(2) Johnson, J. (1925). *Wisconsin Agr. Expt. Sta. Res. Bull.* lxiii.
(3) —— (1927). *Ibid.* lxxvi.
(4) Quanjer, H. M. (1920). *Phytopath.* x, 35.
(5) Schultz, E. S. and Folsom, D. (1923). *Journ. Agr. Res.* xxv, 43.
(6) Smith, K. M. (1928). *Nature*, cxxi, 904.
(7) Vanterpool, T. C. (1926). *Phytopath.* xvi, 311.
(8) Fernow, K. H. (1925). *Cornell Univ. Agr. Exp. Sta. Mem.* xcvi.
(9) Berkeley, G. H. (1927). *Scientific Agriculture* (*Canada*), vii, 210.
(10) Henderson Smith, J. (1928). *Ann. App. Biol.* xv, 155.
(11) Dickson, B. T. (1925). *Science*, lxii, 398.
(12) Hoggan, I. A. (1927). *Journ. Agr. Res.* xxxv, 651.

DESCRIPTION OF PLATES XXIV—XXVI

PLATE XXIV.

Fig. 1. Leaves from tomato inoculated with potato mosaic, showing the spot-necrosis type of symptom.

Fig. 2. Leaves from tomato inoculated with potato mosaic, showing the mottle type of symptom.

PLATE XXV.

Fig. 3. Leaf of *Hyoscyamus niger*, inoculated with potato mosaic. Size of leaf $5\frac{1}{4}$ in.

Fig. 4. Leaf of *Datura stramonium*, inoculated with potato mosaic. Size of leaf $5\frac{1}{2}$ in.

PLATE XXVI.

Fig. 5. Leaf of tobacco (var. White Burley), inoculated with potato mosaic. Size of leaf $9\frac{1}{2}$ in.

Photographs by V. Stansfield.

(*Received May 8th*, 1928.)

Fig. 2.

Fig. 1.

HENDERSON SMITH.—The Transmission of Potato Mosaic to Tomato (pp. 517–528).

Fig. 4.

Fig. 3.

HENDERSON SMITH.—The Transmission of Potato Mosaic to Tomato (pp. 517–528).

Fig. 5.

HENDERSON SMITH. The Transmission of Potato Mosaic to Tomato (pp. 517-528).

"BREAKING" IN TULIPS

By D. M. CAYLEY.

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(With Plates XXVII–XXIX and 4 Text-figures.)

The phenomenon of "breaking" in garden tulips has been known for several hundred years, and the large number of variegated tulips now on the market have originated from, and are due to this peculiar form of what might be called chlorotic variegation, for want of a better term.

It is a well-known fact that when tulips are raised from seed, the flowers for the first few years are of a uniform "self" colour, the colour of course being different according to the variety. These self-coloured seedlings are known as breeders. At any time these breeders are liable to "break." The flowers become variegated to bi-colours, the original self colour or a darker shade of the same being restricted to splashes, stripes, or lines, somewhat irregularly distributed on a white or yellow ground as the case may be.

As a rule a few only of a batch of breeders of a given variety "break" in any one year. Cases are known in which both breeders and "broken" forms of a particular variety have co-existed for seventy or eighty years. The "breaking" affects the anthocyanin red to purple pigment of the tulip, which is confined to the epidermis of the flower, and which becomes segregated into the streaks of the broken flower, allowing, in the patches between, the white or yellow plastid colour of the mesophyll to show through. The orange, scarlet and brown shades of tulips, both "breeder" and "broken," are due to the superimposition of the pink, crimson, or purple anthocyanin in the epidermis over a plastid yellow ground instead of a white one. Broken tulips often show patches also of the unaltered breeder colour in addition to the broken markings and the white and yellow ground colour.

The leaves of the "broken" plants are also mottled or striped irregularly into areas of lighter green.

Thus the active agent which brings about "breaking," whatever it may be, virus or enzyme, can inhibit the formation of anthocyanin sap colour in certain areas in the flowers, and also affect the chloroplasts in

the lighter areas of the leaves. "Breaking" does not seem to diminish the size of the flower, but somewhat restricts the growth of the rest of the plant; the plants are not so tall, and the root system not so well developed (Plates XXVII, XXVIII). Otherwise growth is more or less normal and the bulb can be propagated vegetatively for a number of years without further loss of vigour.

"Unbroken" bulbs multiply much more rapidly than "broken" bulbs.

It is an interesting fact, that no wild species of tulip has been known to "break" either in the wild state or under cultivation; the phenomenon, as far as is known at present, is restricted to garden varieties.

The nature of the active agent which brings about "breaking" is not known. The general appearance of the mottled leaves suggests virus disease, such as mosaic, or possibly some form of infectious variegation or chlorosis (chlorosis infectiosa—Baur(1)) similar to the variegation in *Abutilon striatum Thomsoni*, which can be conveyed from stock to scion by grafting or budding, but is not transmitted in the seed. Walther Hertzsch(2) states that the variegated form of *Abutilon* known as *A. striatum Thomsoni* arose as a single individual in a batch of seedlings of *A. striatum* from seed imported from the West Indies in 1868 by Veitch and Sons, propagated vegetatively on a large scale and then put on the market by that firm. Hertzsch carried out a number of infection experiments, and found that the agent causing variegation, which he calls a virus in *A. striatum Thomsoni*, could be transmitted to other species of Malvaceae, and other varieties of *Abutilon*, but that the form of induced variegation differed with the different hosts. He also found that, if fully expanded variegated leaves are removed and the plant then kept in the dark for a time, the variegation mostly disappears from the leaves that unfold in the dark. When again brought into the light the plant will produce mostly green leaves, and if any leaves showing slight traces of variegation are removed the plant will remain green.

In very susceptible species of Malvaceae the virus increases very rapidly in strong light and heat, so much so, that the plant may die for lack of chlorophyll, whereas in the winter, although variegated, it can survive.

As yet no experiments have been carried out to investigate the effect of light on the "breaking" of tulips, but it is generally held by growers that "breaking" is general on warm soils and most severe in a hot season. In the tulip, however, the plant has sufficient vigour to withstand the detrimental effect of virus, and the relation of virus to host is more or less symbiotic.

With the object of finding out whether "breaking" in tulip is or is not caused by disease, or some such transmissible variegation as described by Hertzsch in *Abutilon*, a series of experiments was started in September 1927.

The results have shown that "breaking" can be transmitted by artificial means from bulb to bulb, when they are in a dormant condition. But the problem as to how and when infection is brought about in nature has yet to be solved. Some work has been done in America at the Oregon Agricultural Experiment Station, and there they claim to have demonstrated that "breaking" in tulips is an infectious mosaic disease spread by an insect carrier.

Although infectious, the "broken" condition in tulips can hardly be considered to be a disease, used in the strict sense, otherwise fixed "broken" varieties would be wiped out in the course of a few years; but it is rather of the nature of a contagious variegation, resulting in a more or less symbiotic condition between host and active agent or virus; presumably the latter can only increase in living tissue during metabolism, and can only exist outside the host plant in tissues of another living organism, possibly an insect.

That the infective agent is present in the bulb itself when in a dormant condition has been proved by these experiments. Also the results suggest that the degree of "break" is proportional to the volume of infected tissue introduced (Plate XXIX, AA′ CC′).

Thus, if infection is carried by sucking insects such as aphis, the amount introduced would be small and the infected area some considerable distance from the young buds in the axils of the bulb scales destined to produce the flowering shoot for the following and subsequent years. Hence the virus might take some time to penetrate, and no immediate effect be produced. On the other hand if infection is carried by bulb-infecting pests such as the millipedes, the infection might be carried directly to the young growing points.

Infection can be localised in the same tulip plant. A "broken" bulb may have "unbroken" offsets, and vice versa; "broken" offsets can occur on an "unbroken" bulb, but the bulb itself when once "broken" remains "broken," although the degree of "breaking" may vary considerably from year to year.

It is alleged by some that "broken" bulbs, in the field, can occasionally revert to the self-coloured breeder from which they arose, but this requires further confirmation under more strictly controlled conditions.

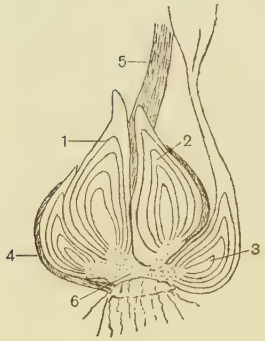
After flowering, the old bulb dies and is replaced by a new bulb from

a bud which is given off from the base of the current year's flowering shoot, and thus is in immediate contact with infected tissue, but offsets are produced frequently, though not always, from buds from scales near the exterior of the bulb. In the case of "broken" offsets in an "unbroken" bulb, the seat of infection is probably in the offset itself (Text-fig. 1).

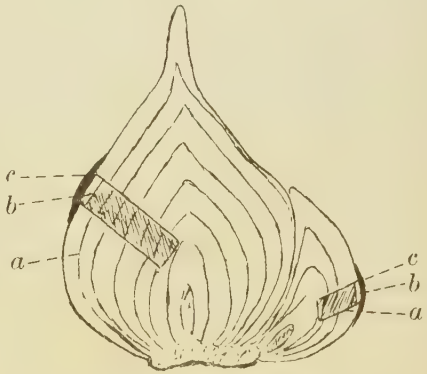
Methods.

The May flowering tulip Bartigon, a self-coloured crimson variety, was used for the experiments, as it is generally held to be a variety which does not "break" very readily. The resting bulbs received direct from the growers were treated in September when in the dormant condition, as follows:

1. Bulbs were plugged with tissue from bulbs which were known to have "broken." The outer brown protective skin was removed and the



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Section through tulip plant, immediately after flowering. 1, 2, flowering bulbs for next year; 3, offset which has produced a leaf; 4, dormant offset; 5, base of flowering shoot; 6, bulb base which will disintegrate, when the bulbs ripen.

Text-fig. 2. Plugged bulb and lateral. *a* = plug of tissue from another bulb; *b* = paraffin wax; *c* = Canada balsam.

surface of the bulb round the point of insertion rubbed over with a swab of cotton-wool soaked in absolute alcohol. A plug was removed from the bulb with a sterile cork borer 5 mm. in diameter, the borer being pushed well down in a slanting direction, so as to get as near the growing point as possible. The plug was removed and with the same cork borer another plug taken out of a "broken" bulb (previously swabbed with alcohol) and pushed down into the healthy bulb with a sterile glass rod. The

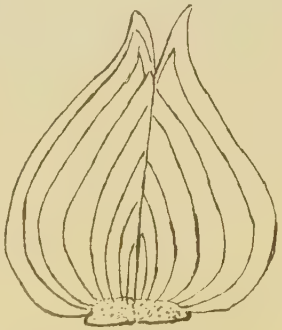
surface was sealed over with melted paraffin wax, and covered with a layer of Canada balsam to prevent evaporation (Text-fig. 2).

The cork borer was dipped in alcohol and flamed between each operation, and thoroughly heated before being used for a fresh experiment. The control bulbs were treated in the same way except that the plug inserted was taken from a healthy Bartigon bulb.

2. A filtrate was made by crushing 300 gm. of "broken" bulb tissue in a pestle and mortar; this was mixed with 300 c.c. of distilled water, strained through muslin and filtered through a Chamberland filter, and used as fresh as possible. Unfortunately the operation of injecting bulbs is a slow process, and the bulbs could not all be treated the same day, so the filtrate was not so fresh for some bulbs as for others. However, the results from the filtrate were negative in all cases.

The filtrate was injected into the bulbs by removing a plug of tissue as above and pouring in approximately $\frac{1}{2}$ c.c. of the filtrate with a sterile pipette. The plug before being replaced was shortened by cutting off the end with a sterile scalpel, thus removing the layers that were nearest the growing point. The rest of the plug was then replaced and sealed up. The bulbs varied as to the amount of filtrate that could be injected, but the majority of the bulbs absorbed about $\frac{1}{2}$ c.c. As control, bulbs were injected with $\frac{1}{2}$ c.c. of distilled water, to see the effect produced by liquid injections.

3. "Broken" bulbs were grafted on to healthy Bartigon bulbs. Whip grafting was attempted but was found impracticable, as it caused too much disturbance and injury. The Bartigon bulb was cut in two, vertically, just avoiding the growing point, and a cut made down the infecting bulb to fit as nearly as possible over the cut in the Bartigon bulb, leaving the growing points intact. The two halves were tied tightly together with raffia previously boiled, and the cut sealed with paraffin wax and Canada balsam (Text-fig. 3).



Text-fig. 3. Two bulbs grafted together.

As a control, healthy Bartigon bulbs were grafted together in pairs in the same way. The whole of the treatment was done in as aseptic conditions as possible. As further controls, some bulbs were planted as received from the growers without any treatment. Another set was peeled before storing as for treated bulbs.

The bulbs were thoroughly dusted over with flowers of sulphur to keep down fungoid growths on the exterior of the peeled bulbs, then stored in shallow trays in single layers, packed in cocoanut fibre. The surface of the fibre was peppered with Keatings' powder, to keep off aphis, and the trays stored six weeks in a cool shed before planting on October 17th. The bulbs were examined before planting, and it was found that both those that had been plugged, and those that had received filtrate had lost turgor and were somewhat soft when pressed, but no case of definite rot was found. During storage about three aphis were found on one bulb, and were removed as soon as detected. The bulbs were planted in separate plots for each treatment, and the controls divided up into four lots, and interspersed between the treated plots. The plots were separated from one another by a 15 in. path.

Results.

1. 50 bulbs plugged with "broken" tissue.

   The plugging caused considerable damage, only 32 plants showed above ground and 15 flowered. The "breaking" was only slight, consisting of a few narrow streaks or splashes of white or paler colour, but nevertheless was quite definite. The "breaking" was determined by examining the inner sides of the petals. The outer sides are apt to be somewhat damaged by rain, wind, etc. The margin of the petals of the "broken" flowers was irregularly serrated (Plate XXIX, AA′ CC′), instead of being almost entire as in the controls BB′.

   6 slight "breaks" in 15 flowers, *i.e.* 40 per cent.

2. 50 bulbs with 1 lateral bulbil—both plugged "broken" tissue. A smaller cork borer 4 mm. diameter was used for the bulbil (Text-figs. 2 and 4) and the bulb plugged on the side away from the bulbil.

   Here also considerable damage resulted.

   5 bulbs and laterals flowered.

   11 bulbs only flowered.

   4 laterals only flowered.

   3 bulbs gave "broken" flowers, two of which are figured in Plate XXIX. No "break" occurred in the laterals.

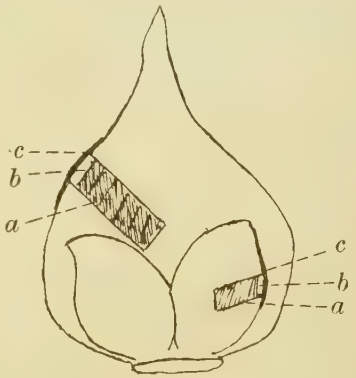
   3 slight "breaks" in 20 flowers = 15 per cent.

3. 50 bulbs with 1 lateral. Lateral only plugged.

   47 plants flowered, but only 2 laterals. No "break" = 0 per cent.

4. 30 bulbs with 2 laterals, bulb and 1 lateral plugged, the other lateral left untreated (Text-fig. 4).

   20 plants showed above ground.



Text-fig. 4. Plugged bulb and one lateral, the other not treated. *a* = plug of tissue from another bulb; *b* = paraffin wax; *c* = Canada balsam.

9 flowered.
5 bulbs only flowered. No "break."
4 laterals only flowered. 2 slight "breaks" and 1 doubtful.
2 slight "breaks" in 9 flowers. Numbers too small, but percentage = 22·2 per cent.

5. 70 bulbs only injected with filtrate.
52 plants above ground.
23 flowered.
No "break" = 0 per cent.

6. 50 bulbs with 1 lateral. Both bulb and lateral injected with filtrate. Considerable damage.
25 plants above ground.
7 sets of bulb and lateral flowering.
15 bulbs only flowering.
1 lateral only flowering.
No "break" = 0 per cent.

7. 60 Bartigon bulbs grafted with 60 "broken" bulbs of the variety Sulphur (Text-fig. 3).
55 plants above ground.
47 { 37 double sets both varieties flowering.
10 Bartigon bulb only flowering.
4 Sulphur bulb only flowering.
2 diseased—no flower.
1 diseased—no flower.
1 double set with a rogue variety not Bartigon, but also "broken."
13 pronounced "breaks" in 47 sets = 27·6 per cent. (Plate XXVII, fig. 2.)

8. 30 Bartigon bulbs grafted Kroeschler—a "broken" variety.
21 plants above ground.
17 double sets, both varieties flowering.
2 Bartigon only flowering.
2 Kroeschler only flowering.
4 pronounced "breaks" in 19 sets = 21 per cent. (Plate XXVII, fig. 1.)

9. 20 Bartigon bulbs grafted with 20 Kaleidoscope—a "broken" variety.
19 plants above ground.
12 double sets, both varieties flowering.
4 Bartigon only flowering.
3 Kaleidoscope only flowering.
5 very pronounced "breaks" out of 16 Bartigon flowering plants = 30·1 per cent. (Plate XXVIII, fig. 3.)

Controls.

(*a*) Control to Exps. 1, 2, 3, 4.
25 bulbs plugged healthy tissue.
18 plants above ground.
12 plants flowering.
No "break" = 0 per cent.

(*b*) Control to Exps. 5, 6.
25 bulbs injected with sterile distilled water.
21 plants above ground.
9 plants flowering, 8 bulb only, 1 bulb and lateral flowering.
No "break" = 0 per cent.

(*c*) Control to Exp. 7.
20 healthy Bartigon bulbs grafted with 20 other healthy Bartigon bulbs, in pairs. 40 bulbs in all.
20 sets above ground.
16 showing 2 Bartigon flowers.
4 showing 1 Bartigon flower.
Number of bulbs flowering—36.
No "break" = 0 per cent. (Plate XXVIII, fig. 4.)

(*d*) Untreated bulbs—planted as received from the growers.
84 bulbs planted.
83 plants flowering.
No "break" = 0 per cent.

(*e*) 115 bulbs peeled but otherwise untreated.
108 flowering plants.
No "break" = 0 per cent.

Over and above the controls already recorded, over 400 untreated bulbs of the same variety Bartigon, from the same firm of growers, were forced in the spring and 3 "breaks" were recorded = 0·75 per cent.
Total number of controls, 648, "breaks" 3 = 0·46 per cent.
The results are given in tabular form on page 538.

The above results show that the percentage of "breaking" in Bartigon can be increased artificially on an average of 26 per cent. during the course of one growing season by means of bringing the internal tissues of an "unbroken" bulb in contact with freshly cut living tissue from a "broken" bulb, when both are in a dormant condition. The degree of breaking appears to be proportional to the amount of infected tissue introduced, the bulbs plugged with small plugs of "broken" tissue showed only slight but quite definite "breaks" (Plate XXIX); whereas two bulbs, one "broken" and the other not, when cut vertically so as to leave the growing points intact, and the halves of each bulb tied together, produced much heavier "breaking" (Plates XXVII and XXVIII).

The slight "breaks" induced by plugging were also accompanied by irregular lobing and splitting of the margin of the petals, an appearance known in the trade as "parrotting," which did not occur in bulbs plugged with healthy tissue, and thus cannot be attributed to injury. Whether this "parrotting" will prove to be permanent or only transitory remains to be seen.

The plugging caused considerably more damage than the grafting, the percentage of weakly growing plants which failed to flower amongst the plugged bulbs was high. The filtrate from "broken" tissue produced no effect other than approximately the same amount of damage as was caused by plugging or injections with sterile distilled water.

This negative result may be attributed to the dilution of the filtrate. On the other hand it may mean that the active agent is not a filter passer, or may be destroyed or weakened by contact with the air. The filtrate was obtained by filtering the freshly extracted sap from the bulb, which was strained through muslin only before being put into the filter. As found by Kraybill and Eckerson(3) with tomato mosaic it may be necessary first to remove the colloidal substances from the juice, in order to enable the virus to pass through the filter. This point requires further investigation.

The "broken" flowers on grafted bulbs differed in no respect from naturally "broken" flowers of other varieties, and the grafted controls developed quite normally showing that the change was not due to injury.

The grafted bulbs do not join up; cutting a bulb in half appears to stimulate the production of bulbils in the axils of the internal scales near the cut surface, so that the two halves get pushed apart during the growing season. The roots however become entangled, and keep the two bulbs more or less in contact at the base.

In conclusion I wish to acknowledge the help of the laboratory assistant E. F. Emarton, in manipulating the bulbs subjected to the various treatments, and also for taking the photographs for the plates.

Summary.

1. "Breaking" in Tulips is infectious and can be induced by bringing the internal tissue of a normal bulb in contact with tissue from a "broken" bulb during the resting stage.

2. The degree of "breaking" appears to be proportional to the amount of infected tissue introduced.

3. The phenomenon of "parrotting" has appeared in the "broken" flowers from bulbs infected with a small amount of "broken" tissue.

4. Injections of filtrate from "broken" tissue have given negative results so far.

Table.

Experiment	No. of bulbs planted	Treatment	Plants above ground	No. of Bartigon plants which flowered: Bulb only flowered	Bulb and lateral flowered	Lateral only flowered	Total	"Breaks"	%
1	50	Bulbs only plugged "broken" tissue	32	13	2	—	15	6 slight	40·0
2	50	Bulbs with 1 lateral. Bulb and lateral plugged "broken" tissue	—	11	5	4	20	3 slight	15·0
3	50	Lateral only plugged "broken" tissue	47	45	2	—	2	0	0
4	30	Bulbs with 2 laterals. Bulb and 1 lateral only plugged "broken" tissue	20	5	—	4	9	2 slight 1?	22·2
5	70	Bulbs only injected with filtrate	52	15	4	4	23	0	0
6	50	Bulbs with 1 lateral. Bulb and lateral injected with filtrate	25	15	7	1	23	0	0
7	60	Bartigon grafted "broken" Sulphur	55	Bartigon only 10	Double sets 37	Sulphur only 4	47	13	27·6
8	30	Bartigon grafted Kroeschler ("broken")	21	Bartigon only 2	Double sets 17	Kroeschler only 2	19	4	21
9	20	Bartigon grafted Kaleidoscope ("broken")	19	Bartigon only 4	Double sets 12	Kaleidoscope only 3	16	5	31·2
Total	410		271	120 only 75 treated	84	13 Bartigon	174 Bartigon	33	19
		Not including filtrate injections which gave negative results throughout and the 45 untreated bulbs in Exp. 3.							
Total	240			45	73	8	126	33	26·3
				Controls.					
a	25	Bartigon bulbs only plugged healthy tissue	18	12	—	—	12	0	0
b	25	Bartigon bulbs injected sterile distilled water	21	8	1	—	9	0	0
c	40	40 Bartigon bulbs grafted in pairs	40	4	Both bulbs = 32	—	36	0	0
d	84	Untreated Bartigon	83	—	—	—	83	0	0
e	115	Bartigon bulbs peeled but otherwise untreated	108	—	—	—	108	0	0
f	400	Untreated Bartigons but forced	400	—	—	—	400	3	0·75
Total	689		670	—	—	—	648	3	0·5

CAYLEY.—"Breaking" in Tulips (pp. 529–539).

CAYLEY.—"Breaking" in Tulips (pp. 529–539).

CAYLEY.—"Breaking" in Tulips (pp. 529–539).

LITERATURE CITED.

(1) Baur, E. (1904). Zur Aetiologie der infektiösen Panachierung. *Ber. d. D. Bot. Ges.* xxii, Heft 8.

—— (1906). Weitere Mittheilungen über die infektiöse Chlorose der Malvaceen, und über einige analoge Erscheinungen bei *Ligustrum* und *Laburnum*. *Ibid.* xxiv, Heft 8.

—— (1907). Über infektiöse Chlorosen bei *Ligustrum*, *Laburnum*, *Fraxinus*, *Sorbus* und *Ptelea*. *Ibid.* xxv, Heft 7.

—— (1908). Über eine infektiöse Chlorose von Euonymus japonicus. *Ibid.* xxvi a, Heft 8.

(2) Hertzsch, W. (1927). Beiträge zur infektiösen Chlorose. *Zeitschr. f. Bot.* xx, Heft 2.

(3) Kraybill, H. R. and Eckerson, S. H. (1928). Tomato mosaic. Filtration and inoculation experiments. *Contributions from Boyce Thompson Inst. for Plant Research*, i, No. 5.

EXPLANATION OF PLATES XXVII—XXIX.

PLATE XXVII.

"Breaking," caused by grafting a "broken" bulb on to a healthy one.

Fig. 1. A. "Kroeschler," a "broken" variety; transmitter. B. "Broken" Bartigon.

Fig. 2. A. "Broken" Sulphur bulb; transmitter. B. "Broken" Bartigon.

PLATE XXVIII.

Fig. 3. A. Kaleidoscope, a "broken" variety; transmitter. B. "Broken" Bartigon.

Fig. 4. A, B. Two healthy Bartigon bulbs grafted together.

PLATE XXIX.

Specimens of slight "break" induced by plugging with tissue from "broken" bulbs—also slight "parrotting" in affected flowers.

A, C. Slightly "broken" Bartigon. B. Unbroken Bartigon control. A′, C′. Same flowers as A and C respectively viewed from above, to show "breaking" and "parrotting." B′. "Unbroken" Bartigon control.

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A TRANSMISSIBLE VIRUS DISEASE OF THE EASTER LILY

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(With Plates XXX–XXXIII.)

CONTENTS.

Introduction.

In the Bermuda Islands, situated in the West Atlantic at about 32° N. lat. and 64° W. long., the Easter lily is grown on a large scale for the production of bulbs, which are shipped to the United States, Canada and Europe, where they are forced in greenhouses to supply flowers for

use at Easter and Christmas, for wreaths, and for other decorative purposes.

The Easter lily is probably a native of the Liukiu Archipelago, a chain of islands stretching from the south of Japan to Formosa and strikingly similar to Bermuda in their equable though humid climate and in their calcareous soil.

The variety grown in Bermuda, the so-called "Lilium Harrisii," apparently reached Belgium from the Orient in 1830, when it was grown in the Botanic Garden at Ghent. It is known botanically as *Lilium longiflorum* var. *eximium*, a name given it by J. C. Baker in 1871. It is considered to be superior to *Lilium longiflorum*, grown in the Azores, Japan and elsewhere, on account of the greater number of flowers it produces, the larger size and finer texture of the blooms and its adaptability to high forcing temperatures.

Clear evidence has recently been brought forward to show that the Easter lily was present in Bermuda as early as 1856. A portfolio of water-colour drawings of Bermuda plants made by a Dr Cogswell, an English botanist, is in the possession of the Bermuda Library and includes an excellent representation of "Lilium Harrisii" in bud and in flower. The drawing is dated April 12th, 1856.

The commercial possibilities of the lily were first realised by General Russell Hastings, a retired civil war veteran, who sent trial shipments of bulbs to the United States. In 1876 bulbs reached the hands of W. K. Harris, a well-known greenhouseman of Philadelphia, who launched them on the trade under the name of "Lilium Harrisii."

Decline of the lily industry.

Careful examination of references to the trade in newspapers and in horticultural journals has shown that in all probability serious disease made its appearance in the Bermuda lily fields about 1893. The maximum production took place in 1896, when 13,803 boxes of bulbs were exported, valued at £13,574. After 1896 there was a rapid decline, only 2322 boxes being exported in 1897. From that date onwards some 6000 boxes were exported per year. By 1913 the number had dwindled to 2357, valued at £3470.

Resuscitation of the industry.

In 1919 the number of cases of bulbs exported was 664. By empirical methods some of the growers were able largely to eliminate disease from their stocks. The discovery of at least one of the factors which led to the

previous failure has enabled the Agricultural Department to take intelligent action, with the result that in 1927 over 6000 cases of bulbs of very fine quality were exported.

The European market, however, was practically entirely lost. The growers had suffered so severely from diseased bulbs in the past that they were unwilling to make a fresh trial of the Bermuda strains. This prejudice still obtains in the United States also, though to a lesser degree. Thus E. H. Wilson writes in *The Lilies of Eastern Asia*, "To-day (the lily industry in Bermuda) is merely the ghost of its former greatness and relatively unimportant" (11).

The European market was seized by Japan and the Azores, Japan exporting *Lilium longiflorum* var. *insulare*, known to the trade as "Lilium formosum," and *Lilium longiflorum* var. *takesima*, known to the trade as "Lilium giganteum," and the Azores exporting a strain of *Lilium longiflorum*. The Japanese hold 90 to 95 per cent. of the European trade in Easter lilies.

Cultivation of the Easter lily in Bermuda.

The cultivation of the Easter lily in Bermuda is practically entirely by vegetative methods. The outer scales of the bulbs are removed and either placed in damp sand or soil throughout the summer or planted directly in the soil in late summer. On the broken areas of the scales small bulbils are formed which produce a flowering plant in the course of one growing season, November to July. Under ordinary circumstances they will produce a bulb some 6 in. in circumference. These bulbs, dug in July, and replanted a few months later, will yield bulbs 9 in. or more in circumference.

The hearts of the bulbs are also used for propagation, as are the "side stock," or small bulbs formed on the stem above the parent bulb.

The plants come above ground in October and usually commence flowering early in March, the main burst of bloom of "Lilium Harrisii" being around Easter time.

The plants die down during June and the bulbs are usually ready for digging in early July. They are carefully cleaned and graded and packed in coral sand in large wooden boxes for export.

Under greenhouse conditions a steady temperature of 60 to 70° F. after the plants are well up is necessary for successful results.

THEORIES REGARDING THE CAUSE OF THE COLLAPSE OF THE INDUSTRY.

The first reference to any detailed investigation of the diseases of the Easter lily in Bermuda may be found in *The Lily disease in Bermuda* by Alex. Livingston Kean(1), published in 1890. This paper refers to the *Botrytis* disease, which had been made the subject of a classical paper by Prof. H. Marshall Ward two years previously. It is not of interest to us in the present connection as it is now evident that the *Botrytis* disease, though present from year to year, was of much importance only in seasons of excessive humidity. The writer appears however to have made a careful study of the plants in the field, and it is noteworthy that he does not refer to any of the symptoms which were in later years to become noticeable in the fields.

As stated above, the first severe onslaught of the disease occurred about 1893. In 1897, Albert F. Woods, of the United States Department of Agriculture, presented a report entitled "The Bermuda Lily Disease"(12). In the letter of transmissal we read, "During the past five or six years a disease, which is apparently becoming more destructive each season, has seriously interfered with the profitable growth of the crop." In this paper, Woods described a disease "characterised by the spotting and distortion of the leaves and usually of the flowers, spotting of the scales of the bulbs, and generally the stunting of the plants." It is now apparent that Woods referred here to two distinct diseases, one of which is that under discussion and the other of which (a mosaic disease) is under investigation at the present time[1]. Woods attributed the disease to a combination of factors: weakening of the plants by improper selection and improper propagation, further increased by the attacks of mites and certain fungi and bacteria. The control measures to be adopted were proper cultivation, selection, and rotation. Premature digging was to be avoided.

In the *Report of the Board of Agriculture* for 1898(15) we read that "the attention of the Board was drawn to the existence of a fungus or mite or both which was becoming very destructive to lilies." The then Director of Agriculture, Mr G. A. Bishop, investigated the matter and presented "A report on the diseases affecting the lily in Bermuda, their cause, treatment, prevention, etc."(1). According to him the trouble

[1] The mosaic diseases of this and other lilies are at present under investigation by the writer and Mr Carl E. F. Guterman, the latter working at the Boyce Thompson Institute, Yonkers, N.Y., in pursuit of a co-operative project by the New York Horticultural Society, Cornell University, the New York Botanical Garden, and the Bermuda lily growers.

was due to various factors, heavy manuring, repetition of the crop on the same ground, reduced vitality, bad selection of stock, and in some cases insufficiency of plant food, "all of which render the bulb prone to an attack of fungi or soft rot. When the fungi or rot has taken possession of the bulb, it causes the roots and base to become rotten, after which it is liable to become attacked by the Eucharis mite." Various nostrums were suggested for the control of the disease.

By 1900 some good effects had apparently accrued from the policy of selection recommended by Bishop, but the cause of the disease not being properly understood it still remained a continual menace. In the Report for 1900 (16) we read, "Excess of water in the soil asphyxiated the roots which were immediately attacked by fungus diseases, with the result that *the top leaves became curled and spotted* while the bulbs were free from both animal and vegetative organisms," and further, "*Curly top* and spotted foliage clearly proved that the trouble lay at the root and base of the bulb."

In 1901 Woods published some further remarks on the disease in the *Yearbook of the United States Department of Agriculture* for that date (13). At that time he still supposed that the disease was due mainly to the use of "unripened and unrested bulbs."

In *Country Life of America* for 1904, we read of a visit to Bermuda by Prof. Bailey (17). According to him "the stock became mixed and debilitated and the market lost confidence." He describes the methods of one grower, George W. West, who had apparently had some success despite the prevalence of disease. "His fundamental purpose is to discard all mixed and weak stock, and to collect from here and there such bulbs as represent vigor, healthfulness, and trueness to type, and these bulbs he is planting for the production of his crops."

In the year 1915 a number of lily bulbs were sent to the United States Department of Agriculture by Mr E. J. Wortley, the then Director of Agriculture in Bermuda. Plants from these were grown under greenhouse conditions in Washington. Photographs of the bulbs and plants are in the files of the Bermuda Department of Agriculture. Notes made by C. W. Carpenter indicate that of the plants grown from 137 miscellaneous bulbs, 27 were unmarketable. Nineteen of these 27 are referred to as "yellowed, poor and stunted." None of these plants produced any flowers. The average height of these plants was 2 in., while the average height of the healthy plants was about 23 in. It is clear from the notes and the photographs that there were two diseases present, one a mosaic disease, that mainly described by Woods, and another disease, the effects

of which were even more disastrous and which was characterised principally by very marked stunting.

THE DISEASE.

The leaves of a normal plant of *Lilium longiflorum* are somewhat dark green in colour and curve downwards only slightly. The leaves of "Lilium Harrisii" (*L. longiflorum* var. *eximium*) curve downwards to a somewhat greater degree (Pl. XXX, fig. 1).

It had been noticed for many years by one of the most experienced growers in Bermuda, Mr Howard E. D. Smith, of St David's Island, that in certain plants in his fields the leaves were very markedly curled downwards and pale in colour and that this condition was apparently contagious. He was in the habit of roguing out such plants, to which he gave the name of "yellow flat." He himself is now of opinion that this disease was the main factor in the breakdown of the industry.

The photographs, already referred to, taken at Washington in 1915, show clearly several examples of this disease. (Plate XXXI, fig. 5, is a copy of a photograph taken on April 2nd, 1915, by W. A. Orton. It shows two of a group of five plants grown from bulbs sent by Mr H. E. D. Smith, and said to be affected with the "yellow disease." It is clear from the notes and photographs that four of the five bulbs produced "yellow flat" plants.

The writer was appointed to the post of Plant Pathologist at Bermuda in September, 1923. The disease first came markedly to his notice on February 5th, 1925, when a grower called his attention to a large patch of peculiar appearance in his lily field (Plate XXXI, fig. 6). He stated that some weeks earlier the plants had been infested with insects, which from his description were a species of aphis. The disease was apparently the same as that already noticed by Smith and observed by the writer in Smith's fields.

Since from the information laid before the Board of Agriculture by the writer the disease appeared to be a serious one it was decided that an official inspection should be made of all the lily fields in the islands. This was done, and it was found that it occurred in certain strains to the extent of over 50 per cent. of the plants(7).

It was soon apparent that the disease probably belonged to the group of transmissible virus diseases. The following aspects suggested this:

1. Its very marked prevalence in certain strains or stocks, the growers of which were not in the habit of removing diseased plants.
2. Its apparent connection with an aphis.

3. The symptoms, which resembled somewhat certain diseases of the virus group.

The disease has now been under observation since 1925, and the information gathered since then has proved that the theory was correct. A short note regarding the disease was published in *Nature* in April, 1927 (8).

Symptoms on plants grown from affected bulbs.

Of 42 bulbs from marked plants showing current season infection early in June, 1926, dug on July 5th, 1926, and planted in pots in virgin soil, 40 diseased plants resulted, while two were doubtful. The symptoms of the disease on these plants were as follows:

Leaves, especially the upper and youngest leaves, very markedly curled downwards; not markedly shorter in length than the normal leaves, but on account of the downward curling reaching outwards to only about one-half the distance from the stem of that reached by healthy leaves on a healthy plant. In some cases leaves twisting sideways and somewhat distorted. Leaves not forming a shallow trough as in normal plants, but the upper surfaces flat or slightly convex in cross section. Colour of leaves slightly chlorotic, but without streaks or spots. The general appearance of the plant a flat rosette or cylinder in contrast to the pyramidal shape of the healthy plant (Plate XXX, figs. 2, 3).

Current season symptoms.

Current season symptoms, *i.e.* symptoms produced on a healthy plant by transference of the disease, are very similar to the above. The mature healthy leaves are not visibly affected by the disease, but the fresh growth shows the characteristic symptoms. In the case of plants infected late in the season the leaves tend to be twisted from side to side and the internodes tend to be longer than in the case of typical yellow flat plants. This effect is probably due mainly to high temperatures. The topmost leaves are often extremely twisted (Plate XXXI, fig. 7).

Delayed appearance of symptoms.

An interesting point with regard to plants grown from infected bulbs is the delayed appearance of symptoms. Thus diseased bulbs planted on November 3rd, 1926, produced shoots in the course of about a fortnight. The first leaves to be produced were in most cases however apparently healthy and the typical symptoms did not appear till the middle of December. This phenomenon is very apparent also in the fields, where a crop containing many infected bulbs may look apparently healthy at

the beginning of the season, and later develop the typical symptoms, and is similar to what occurs in the case of leaf-roll of potatoes. The lower leaves usually show a certain amount of downward curling subsequently.

TRANSMISSION EXPERIMENTS.

Preliminary experiments during May and June of 1926 suggested that the disease was transmitted by the aphis most commonly found on lilies in Bermuda, namely, *Aphis gossypii* Glover.

On December 9th, 1926, nymphal stages of this insect were transferred from a yellow flat plant from the Station plots, on which they had been for at least 10 days. The aphids, to an average number of 6, were transferred to 10 healthy plants in pots kept under cages covered with cheesecloth. On January 21st, 1927, about 6 weeks after the transfer of the aphids, 6 of the plants showed on the young leaves the marked curling characteristic of yellow flat. On February 1st, 9 plants out of 10 showed the characteristic curling. Check plants under another cheesecloth cage alongside remained healthy.

On December 17th, 1926, nymphal stages of the insect were transferred, to the number of 2 per plant, from a typical yellow flat plant, to 82 plants in the field. The plants were covered over entirely for 2 days and subsequently dusted with nicotine dust. On January 12th, almost 4 weeks from the time of transfer of the aphids, marked symptoms occurred on the young leaves of 7 of the plants in the first row and 13 in the second row. In the check rows on either side there was found one yellow flat out of 48 and one out of 46 plants respectively.

On December 17th, 1926, nymphal stages of the insect were transferred, to the number of 2 per plant, from a yellow flat plant grown from an infected bulb to 11 healthy plants of *Lilium longiflorum* in the Station beds. The plants were covered over entirely for 2 days and subsequently dusted with nicotine dust. On February 1st, 17 days after the transfer, all of the 11 plants showed a chlorotic appearance of the top and characteristic curling and twisting of the upper leaves. Control rows on either side remained healthy.

On January 26th, 1927, nymphal stages of the insect were transferred, to the number of 5 per plant, to 8 healthy plants in the field. The plants were then on the average 1 ft. in height. All the plants were covered over with a cheesecloth cage. The characteristic symptoms—a chlorotic colour of the leaves, accompanied by curling and twisting—became apparent on the topmost leaves of the plants on the following dates: February 16th, 1 plant; February 18th, 2 plants; February 20th, 4 plants; February 26th, 1 plant.

The first symptoms were manifest three weeks after the date of transfer. Healthy plants alongside remained unaffected.

On January 15th, 1927, 11 healthy plants in pots were placed under a cheesecloth cage, and among them 3 yellow flat plants badly infested with *Aphis gossypii*. By February 1st, 17 days from the date of transfer, all the 11 plants showed the first symptoms of yellow flat on the topmost leaves (Plate , fig. 8).

On December 28th, 1927, 6 aphids from a diseased plant were transferred to each of 6 healthy plants in the field under a cheesecloth cage. On January 24th, 27 days after the date of the transfer, one plant showed distinct evidence of infection on the topmost leaves, while on January 30th, 5 plants out of the 6 showed clear evidence of infection. The remaining plant showed evidence of infection about a week later (Plate XXXI, fig. 9).

On January 5th, 1928, 6 aphids from a diseased plant were transferred to each of 6 healthy plants in the field under a cheesecloth cage. On February 7th, 33 days after the transfer, 3 plants out of the 6 showed distinct evidence of infection.

Field observations seem to indicate that only *Aphis gossypii* is concerned in the spread of the disease; experiments were carried out however with other insects found on lilies in Bermuda.

The only other aphis at all common on lilies is a large brown species, resembling *Aphis gossypii* described by Mr F. E. Theobald as a new species under the name *Aphis Ogilviei* Theob.[1] This species appears to occur in certain fields where *Aphis gossypii* is noticeably absent. It has not been found on any other cultivated or wild plant. No positive results can yet be reported for the transmission of yellow flat by this insect.

The large green aphis *Macrosiphum gei* Koch. (*M. solanifolii* Ashm.) is not infrequent on lilies when they are grown in proximity to potatoes, on which it is known to be a vector of leaf-roll and mosaic. It is found on lilies during the first few months of their growth and again towards the end of the growing season. It occurs commonly on lilies when grown in greenhouses in the United States. In Bermuda its host plants include, besides potatoes and lilies, roses, ranunculus and other ornamentals, and also lettuce. Its favourite wild host is *Sonchus oleraceus* L. Experiments during seasons 1926–27 and 1927–28 indicated that the disease is not carried by this insect.

The aphis *Neotoxoptera violae* Perg. was found on one occasion on

[1] See *The Insects of Bermuda*, by L. Ogilvie, published by the Crown Agents for the Colonies, 1928.

lilies. Attempts at transmission of yellow flat by this insect gave negative results.

Experiments with the mealybug *Pseudococcus citri* Risso, not uncommon on the bases of bulbs which have been stored carelessly and also occurring in Bermuda on potato shoots and on various ornamental plants, also gave negative results.

Numerous attempts at artificial transfer of the disease have been made by rubbing leaves of healthy plants with crushed leaves of yellow flat plants and by injection of filtered juices from leaves of yellow flat plants into the leaves and stems of healthy plants, but with no success.

There is no evidence that the disease is carried in the soil. Bulbs from healthy plants grown in fields badly infested the previous season have produced healthy plants. Again, diseased and healthy plants have been frequently seen growing in the same pot.

THE INSECT VECTOR.

Specimens of the aphis were examined by Mr F. V. Theobald and were at first thought to be identical with *Aphis lilii* Takahashi. Further investigation showed however that it could not be separated from *Aphis gossypii* Glover, a species which has been much confused in collections and in literature on account of its variation in colour and size, its wide geographical distribution, and the large number of its host plants.

On the lily the nymphal stages of the aphis are pale lemon yellow to pale green in colour, with head and cauda greenish and cornicles and eyes black. The first and second segments and tips of the antennae, the apices of the tibiae and the tarsi are dusky.

The wingless viviparous female is yellowish with blackish green mottling, the eyes and cornicles being black and the antennae pale brownish. The first and second antennal segments, the apex of the fifth and the sixth are dark. The cauda is blackish green, the legs pale brownish, the apices of the femora very slightly dusky. The apices of tibiae and tarsi are black.

The body of the winged viviparous female is shining, practically hairless. Head, thoracic lobes and cornicles are black, the antennal segments dark, the apices of femora, apices of tibiae and tarsi dusky, the abdomen greenish yellow to pale green.

Both winged and wingless forms are found throughout the year.

Aphis gossypii is extremely common on the hibiscus hedges, which are a notable feature of the Bermuda vegetation, infesting both the leaves and the flower buds. During the summer months it sometimes

brings about considerable defoliation. It is also found on other ornamentals, such as the flowers of *Zinnia* and *Justicia*, and on certain weeds such as *Plantago major*. The form occurring on hibiscus has been bred successfully on lilies.

Other species of lilies are but rarely grown in Bermuda, but when they are infestation with the aphis commonly takes place. *Lilium candidum*, *Lilium speciosum* and *Lilium testaceum* have been found heavily infested. No other bulbous plants are apparently favoured by the aphis.

In addition to frequenting these other plants the insect tides over the summer on discarded bulbs which have been left lying about the fields or round the packing sheds or which have been stored carelessly in open boxes. It is found especially on bulbs which have become green owing to exposure to the sun. Here, among the outer scales, near the tips, it may sometimes be found in great numbers. Winged forms are not common on the scales.

In the fields the aphis may be found soon after the plants come above ground. In 1925 it was first found on December 11th. In 1926 the winged stages were observed as early as November 29th, and in about the second week in December the aphis was common all over the islands.

In the field the insect congregates chiefly on the young leaves in the centres of the rosettes, but may also be found not uncommonly underneath the old leaves. The latter position is especially common in the case of lilies grown in greenhouses.

The injury done by the aphis itself is slight. When it is especially numerous the excretion of "honey dew" causes the appearance of a sooty mould over the surfaces of the leaves. Under such circumstances also some slight necrosis and slight distortion of the leaves may take place.

The aphis is kept down very efficiently however by several natural enemies. Chief among these is the Braconid *Lysiphlebus* (*Aphidius*) *testaceipes*, Cress., a well-known parasite of aphids, which is also found in Bermuda on *Aphis nerii* Kalt., and *Aphis pseudobrassicae* Davis.

As in the case of these other aphids, *Aphis gossypii* becomes extremely numerous soon after its first appearance on the lilies. The parasite then puts in its appearance and within a few weeks the numbers of the aphids are greatly reduced. In 1926 the first case of parasitism in the fields was seen on December 20th. By January 18th, 1927, few living aphids were to be seen and the straw-coloured swollen bodies of the insects with the exit hole of the parasite were common. The same phenomenon was observed in 1927–28.

With the coming of the warmer weather in March and April the aphis

again becomes somewhat numerous and is not uncommonly seen on the flower buds in dark clusters.

Larvae of the Syrphid fly *Allograpta obliqua* Say., and to a less extent *Mesogramma* (*Toxomerus*) *marginata* Say., are efficient predators on the aphids. Adults of the former are especially common hovering around aphid-infested lily heads in sunny weather.

The Coccinellid beetles *Coccinella munda* Say. and *Scymnus terminatus* Say., larvae of lacewing flies (*Chrysopa* spp.) and the predatory bug *Triphleps insidiosus* Say. (*Anthocoridae*) are also valuable in controlling the aphis during the warmer months.

The disease in the field.

As has been stated above, the first symptoms of the disease in the field do not appear till about the middle of December. It is clear from observations that a great deal of spread takes place during the second half of December, when the aphids are most numerous. The symptoms of spread show up about the middle of January.

Affected plants usually occur in patches in the fields (Plate XXXI, fig. 10). At about the centre of each patch is situated a very stunted plant which has grown from an infected bulb and, at the time of flowering, plants showing current season infection to the number of about 15 or so may be found in a circle surrounding it. In certain fields and in certain seasons, however, the spread may be much more disastrous and the writer has seen large fields infected with yellow flat from a few plants.

Spread may take place right up to the time of flowering. It is difficult to tell the symptoms at the time when the flower buds are beginning to appear since there is naturally a considerable amount of twisting of the topmost leaves round the buds.

Some two months elapse between flowering time and the dying down of the plant. Although the leaves on the main stem are during that time all mature it is very probable that they may still become infected, although infection will not show up until the following season if no side shoots are produced. This is important from the point of view of control. Fields from which all apparently diseased plants had been rogued out yielded bulbs a considerable percentage of which were diseased.

In every case under observation side shoots arising from infested plants showed infection, though here too the typical symptoms did not appear on the lowest leaves but on those some inches up the stem.

Several hundreds of scales from yellow flat bulbs planted in the open ground under a cheesecloth cage yielded young plants in which the yellow

flat symptoms were clearly seen in the slight distortion of the first leaves and typical curling of those subsequently produced (Plate XXXIII, fig. 18).

During late March or early in April, about flowering time, the lower leaves of yellow flat plants turn yellow and the plant dies off rapidly from the base upwards. This phenomenon also occurs in plants affected with the mosaic disease. In the case of healthy plants dying off does not commence till June. Plate XXXII, fig. 11, is a photograph of a badly infected field taken in May, 1925, and shows well the appearance of affected plants at that time of the year.

In this connection it should be mentioned that Prof. H. H. Whetzel, of Cornell University, who acted for a time as Plant Pathologist in Bermuda, made the following observations in June, 1921: "It was noted in all the fields visited that there was a marked difference in the time of dying down of the individual plants side by side in the same bed. This difference was not to be explained by differences in soil, soil moisture, or other similar factors. Certain plants would be green and healthy with no dead leaves even at the bottom of the stalk, while right beside them would be plants the leaves and stems of which were completely dead and dry, other plants near by would be partially dead, *i.e.* the lower leaves would be dead with dying yellow leaves above. In short all stages from completely and evidently long dead plants through plants in various stages of dying up to completely green and healthy plants were to be observed in all the fields. The relative percentage of dead and healthy plants varied in the different fields, but in no case was a field observed in which all the plants were green or all dead." A large number of marked diseased bulbs planted by Prof. Whetzel yielded diseased plants, the difference between these plants and those from healthy bulbs being most striking.

Various symptoms and effects of the disease.

Examination of the roots of yellow flat plants at the time when the leaves are dying off shows that in many cases, though not invariably, they are hollow. This condition is associated with a fungus which is present also in the roots of healthy plants. Its association is mycorrhizal in nature, for in the inner cells of the root coralloid clumps are formed which undergo dissolution. When the growth of the plant has been severely checked by the yellow flat virus this fungus apparently makes considerable inroads on the roots of the plant.

This fungus is followed by the bulb mite *Rhizoglyphus hyacinthi* Banks., a common inhabitant of Bermuda soils, which burrows up the

decaying roots into the base of the bulb, causing the condition known generally as "basal rot." As stated in the writer's 1925 report (7), p. 52, it is not considered that the mite is usually the cause of primary injury to lily bulbs. In plants infected by yellow flat and mosaic however a large percentage of the bulbs are mite-infested. In this connection reference should be made to the remarks of Bishop quoted on p. 544.

As has been already stated, one of the most notable symptoms of the disease is the shortening of the internodes. Under circumstances which tend to produce spindly plants, for example, when tall weeds have been allowed to grow up round the plants or in shady situations, the shortening of the internodes is naturally not so marked and it is sometimes difficult to pick out diseased plants, but the characteristic curling or twisting will usually betray them to the experienced eye.

The disease has a marked effect in stunting the growth of the plant and in reducing the number of buds. This will be clearly seen from the following tabular data obtained on April 4th, 1927.

No. of plants	Date of aphis transfer	Date of first symptoms	Average height in.	Average height of controls in.	Average number of buds
31	Infected 1925	—	4·2	—	0·3
37	Infected summer 1926 (previous season)	—	5·3	—	0·5
25	Dec. 17, 1926	Jan. 12, 1927	7·6	17	1·8
11	Dec. 20, 1926	Jan. 14, 1927	13·7	24	3·4 (longiflorums)
12	Jan. 22, 1927	Feb. 9, 1927	12·2	18	3·3
8	Jan. 26, 1927	Feb. 16, 1927	21	29	5·9

The average number of buds on healthy plants from bulbs 7 to 9 in. in diameter is 6.

Plate XXXII, fig. 13, taken in February, 1928, shows a group of plants grown from the bulbs of plants infected by transfer of aphids in December, 1926. On the left is a healthy plant of the same age. The diseased plants have reached their full height, an average of $2\frac{1}{2}$ in., the tips of the shoots ending blindly.

Plate XXXII, fig. 14, is a photograph of a plant infected in season 1925-26, taken in February, 1928. Such plants are also shown in the foreground in Plate XXXII, fig. 13. It will be seen that the first three or four leaves to appear are somewhat normal in shape, though usually somewhat contorted. The subsequent leaves to appear show the characteristic pronounced downwards curling and chlorotic cast. Only some dozen leaves are produced in all, the shoot ending blindly at the tip and the whole plant being only 1 in. or so in height.

It will be seen that infection with the virus has a progressive effect in reducing the height of the plant and the number of the buds. Bulbs from plants infected the previous year or two years before often do not produce any flower. Even in cases of current year infection the buds are noticeably shortened and are sometimes somewhat blistered, while very frequently the pedicels are turned stiffly downwards in a peculiar manner (Plate XXXI, fig. 7).

Effect on the bulbs.

The effect of the disease on the size and shape of the bulbs is very marked. In the case of current season infection the tendency is to produce a bulb which greatly resembles a bulb of a poor type of *Lilium longiflorum*. The outer scales remain normal and loose; the inner, produced subsequent to infection, are tightly drawn together. The circumference of the bulb is smaller than that of a normal bulb. The height is usually reduced owing to the reduction in height of the inner scales. A very similar general effect can be produced by cutting off the stem some inches above the ground around flowering time.

Another very noticeable effect of the disease is a tendency towards splitting of the bulbs. The result is that there is a reduction in the size of the bulbs from year to year, till the resulting bulbs are little larger than peas.

The following table gives details of the size of the bulbs, etc., resulting from infection at different dates:

No. of plants	Date of aphis transfer	Average circumference of bulbs when dug in.	Average height of bulbs when dug in.	% doubles	% basal rot
39	Infected season 1925–26	3·4	0·75	Practically nil	60*
19	Dec. 17, 1926 (first symptoms on Jan. 12, 1927)	6·75	2	40	80
6	Jan. 26, 1927 (first symptoms on Feb. 16, 1927)	6·6	1	50	100†

* Plate XXXIII, figs. 15, 16. † Plate XXXIII, fig. 17.

Bulbs of an average circumference of 6 in. when planted were grown as checks. When dug these were of an average circumference of 9 in. and an average height of 2 in. They were practically free from doubles and basal rot.

Even in the case of yellow flat plants as small as that shown in Plate XXXII, fig. 14, there is still a tendency for the bulbs to split up. Thus one of the most conspicuous effects of the diseases is the diminished size of infected plants and bulbs from year to year. The practical result in the past was that growers found that their stocks became entirely useless in the course of a few years.

Possibility of recovery.

The writer is of opinion that lily plants once infected never recover from the disease. This is borne out by the experiments recorded above and is in agreement with the behaviour of plants affected with other virus diseases. Cases of apparent recovery are due to masking by shading or by high temperatures.

Behaviour under greenhouse conditions.

Under greenhouse conditions, at temperatures of about 70° F., such as are used in forcing the plants into bloom, the internodes of the diseased plants are lengthened considerably and the leaves tend to twist rather than to curl. The writer paid a visit to greenhouses in Philadelphia, Washington and New York during the winter of 1926–27. The average percentage of yellow flat occurring among Bermuda lilies there was about one, but counts of 10 and 14 per cent. were got in one house. The diseased plants were recognised easily by their pale cast and curled or twisted leaves. The flower buds do not usually develop to any extent but wither off. Growers were familiar with the symptoms and stated that there had been a marked decrease in the amount of the disease during the last few years.

It is thought that spread of the disease takes place in the greenhouses. Cases were seen in which curling and twisting did not occur till 9 in. from the base of the plant. Mr C. E. F. Guterman informs me that he has found *Aphis gossypii* in certain greenhouses. The commonest aphis on lilies in greenhouses in the United States is however *Myzus circumflexus* Buckton.

Occurrence on other lilies.

The disease has been observed on plants of "Lilium formosum" and "L. giganteum" in the greenhouses, the plants being apparently from infected bulbs. Japanese bulbs of "Lilium formosum" planted in an isolated position in Bermuda produced severe yellow flat plants in 3 cases out of 5. "Lilium giganteum" has been infected in Bermuda (Plate XXXII, fig. 12). Photographs of *L. Batemanniae* apparently affected by the disease have been sent me by Mr Guterman.

Careful search in the lily fields of Bermuda has failed to reveal symptoms suggesting yellow flat on any of the common weeds. The nature of its spread too does not suggest the presence of any other host besides the lily.

Mosaic diseases are known from some 15 different species of plants in Bermuda, but from their nature none is supposed to be in any way connected with yellow flat except possibly aster yellows, a disease recently transmitted by L. O. Kunkel to more than 70 species in 28 different families of plants by means of the leafhopper *Cicadula sexnotata* Fall. Aster yellows was first identified in Bermuda by the writer in January, 1927. It occurs very frequently in plantings of lettuce and on marigolds in gardens. The leafhopper is extremely common. The writer's attempts to transmit aster yellows to lilies were unsuccessful, and lilies growing near badly infected plantings were unaffected with yellow flat.

Relation to other diseases of the lily, and similar symptoms produced by other causes.

It has already been shown that the roots of yellow flat plants are liable to be infected by fungi and subsequently by the bulb mite which brings about "basal rot."

Fasciated plants are not uncommon in the fields, especially amongst vigorous strains. The disease is not infrequent on such plants.

Other conditions bring about symptoms liable to be confused with yellow flat. Poor soil produces a stunted, markedly pyramidal growth. Certain soils produce a peculiar twisting of the leaves unaccompanied by a pale cast or by stunting. Bulbs from such plants produce healthy plants the following year. Patches of bright yellow chlorosed plants are often found in the fields, especially after heavy rainstorms. Bulbs from such plants have been known to produce healthy plants the following year when planted elsewhere. Yellow flat has been found on chlorosed plants.

Plants the bulbs of which are affected with severe mite injury ("basal rot") are markedly stunted and somewhat pale in colour, but the leaves have not the characteristic curling of yellow flat plants.

Yellow flat has been transmitted experimentally to mosaic plants. The yellow flat symptoms tend to obscure those of mosaic, the leaves becoming uniformly pale in colour and dying off early. It should be noted here that in a severe type of lily mosaic there is also extreme curling of the leaves, as in yellow flat, but accompanied by very marked striping. This disease has not yet been transmitted experimentally.

PROBABLE INTRODUCTION INTO BERMUDA.

It is known that about the year 1893 Japanese bulbs were imported into Bermuda in large quantities and that later on discarded bulbs were sent down from greenhouses in the neighbourhood of New York. The writer believes that the disease was probably introduced at that time.

Bishop refers thus to the presence of aphids in 1898(1): "Insect Pest: Should the lily become infested with insects, spray with a solution made up of the following: (Quassia, soft soap, etc.)."

CONTROL.

The control of the disease in the field is a simple matter if the stock is not badly contaminated with infected bulbs. The grower should acquaint himself thoroughly with the symptoms of the disease and should make a systematic examination of his fields every few weeks. As soon as diseased plants can be seen, about the middle of December, they should be removed, bulbs and all, and destroyed. If the plants are left lying at the sides of the field, a common practice in Bermuda, they tend to become badly infested with aphids, which spread the disease to the plants in the field.

If the stock is badly infected with the disease it should be destroyed and a start made with a new stock, or healthy plants may be marked at the end of the season and a fresh start made with these, a sharp look out being kept for the appearance of the disease.

The fields may be kept free from aphids by dusting the plants during dry sunny weather with nicotine dust, or by spraying them with a nicotine solution such as "Black-Leaf-40" at a dilution of about 1 in 1000 ($\frac{3}{4}$ pint to 100 gallons), with the addition of 3 to 5 lb. of soap. This solution without the soap may be used in combination with Bordeaux mixture.

In addition to these measures the grower should of course employ commonsense sanitary precautions. No discarded bulbs should be left lying about over the summer, nor should patches of lilies be left undug.

On account of the presence of disease in the fields official inspection was commenced in 1925 and has been carried out by the writer and field assistants in 1926, 1927 and 1928. Counts are made of the percentage of yellow flat, mosaic, basal rot and off-type plants in three parts of the field. The percentages of diseased plants allowed have been cut down year by year. In 1928 the regulations call for entire freedom from yellow flat. The improvement in 1927 and 1928 has been very marked, and it is

thought that if the growers can all be got to remove diseased plants as soon as the symptoms are visible and to destroy them the disease may be entirely eliminated from Bermuda.

Comparison with other virus diseases.

Yellow flat is of interest because it is apparently the first virus disease of the "rosette" type to be described from bulbous plants and apparently the first disease of bulbous plants the vector of which has been determined.

A number of virus diseases are known which produce rosettes from the shortening of the internodes, for example, rosette of ground-nuts, recently described by Storey (10), and shown to be transmitted by *Aphis leguminosae* Theo. Here however there is apparently mottling of the leaves.

Peach rosette, which is probably a virus disease, but the vector of which is not known, is also similar in the shortening of the internodes. Here the pale colour of the leaves reminds us of yellow flat.

Bunchy top of bananas, transmitted by the aphid *Pentalonia nigrinervosa* Coq. is also similar to the shortening of the internodes. In this case too there is early dying off of the roots from the effects of the disease (14).

In wheat rosette (6) we have marked rosette formation and diseased plants usually die off early.

The degeneration of the lily stocks is strikingly parallel to the degeneration of potatoes, which has been shown to be due (as in lilies) to two transmissible virus diseases, leaf-roll and mosaic. Yellow flat resembles leaf-roll in the absence of striping, in the curling and chlorotic colour of the leaves, and in the small size of the bulbs and tubers produced. The methods to be employed for its control are also strikingly parallel.

Mosaic-like diseases appear to occur on numerous bulbous plants, but none has been described in great detail nor apparently has the transmitting agent been described in any of them. A mosaic disease of hippeastrum has been described by Kunkel (5), while Griffiths has referred to mosaic-like diseases of narcissus (2) and tulips (3). Mosaic probably occurs also on hyacinths (9). The writer has observed a mosaic-like disease of irises, particularly *Iris tingitana* and *Iris imperati*, in greenhouses in the United States, while a mosaic of paper white narcissus appears to be causing rather severe losses in that country[1].

[1] A mosaic disease of gladiolus, apparently associated with *Aphis gossypii*, has recently been described by Miss Louise Dosdall (*Phytopathology*, XVIII, No. 2, Feb. 1928, p. 215). See also Dr Dorothy M. Cayley's paper on the "breaking" of tulips in this issue.

SUMMARY.

1. A summary is given of the history of the Bermuda Easter lily (*Lilium longiflorum* var. *eximium* Baker), known to the trade as "Lilium Harrisii."

2. It is known that about 1893 "a peculiar sickness" appeared in the lily fields of Bermuda, which caused a very marked decline in the yearly amount of bulbs exported from the Colony.

3. Subsequent to 1919 a resuscitation of the industry has taken place, and especially in recent years, when the chief cause of its failure has been ascertained.

4. A summary of the methods of cultivation in Bermuda is given.

5. Theories as to the cause of the disease were put forward by Woods, Bishop, and others. It is shown that there were two types of disease, one resembling a mosaic, the other characterised principally by very marked stunting and downward curling of the leaves. The latter is here dealt with.

6. Photographs taken in the United States Department of Agriculture greenhouse at Washington in 1915 show clearly examples of the disease. The disease came prominently to the notice of the writer in 1925 when certain aspects of it suggested that it was a virus disease. It was called "yellow flat" by the grower who first observed it.

7. The general appearance of a plant grown from an infected bulb is a flat rosette or cylinder. The leaves are very markedly curled downwards and are slightly chlorotic in colour, but without streaks or spots.

8. In current season infection the leaves which are mature at the time of infection do not show the symptoms. The young leaves show considerable twisting besides curling.

9. Plants from affected bulbs do not show the symptoms till about 4 weeks after coming above ground.

10. Transmission experiments are described in which positive results were secured by the use of *Aphis gossypii* Glover. Experiments with the following were unsuccessful: *Aphis Ogilviei* Theob., *Macrosiphum gei* Koch., *Neotoxoptera violae* Perg., and *Pseudococcus citri* Risso. Attempts at mechanical transfer were also unsuccessful. There is no evidence that the disease is carried in the soil.

11. *Aphis gossypii* is described. Its biology is discussed. It is parasitised by *Lysiphlebius* (*Aphidius*) *testaceipes* Cress. and attacked by certain Coccinellids, larvae of Syrphids, etc.

12. Spread of the disease takes place mainly early in the season.

Affected plants occur in patches in the fields, the average number of infected plants round a bulb-infected plant being about 15.

It appears that the disease is transmitted to the scales and side shoots of affected plants.

Affected plants begin to die down about flowering time, about two months before healthy plants.

The root system is affected and is attacked by secondary organisms, fungi and the bulb mite.

Bulbs from affected plants are flat, small and compact, and resemble bulbs of poor types of *Lilium longiflorum*.

There is a marked tendency to splitting of the bulbs, so that in successive years smaller and smaller bulbs are produced. Amongst weeds or in shady situations the affected plants are not so markedly stunted.

Plants from affected bulbs seldom produce flowers. In current season infection the number of flowers is considerably reduced, the flowers twisted and blistered and the pedicels turned stiffly downwards.

13. In the writer's opinion recovery from the disease never occurs.

14. At temperatures of about 70° F., under greenhouse conditions, the internodes are often lengthened and the leaves twisted rather than curled.

15. The disease has been observed on "Lilium formosum" and "Lilium giganteum" and apparently occurs in Japan. It is apparently not connected with any virus diseases of other plants occurring in Bermuda.

16. Similar symptoms brought about by other causes are described.

17. The disease was probably introduced into Bermuda from Japan about 1893, either direct or via the United States.

18. The means of control recommended are roguing, spraying with contact insecticides, and clean cultivation.

The official inspection carried out by the Government since 1925 has already reduced the disease to a practically negligible quantity.

19. The disease is compared with other virus diseases. It is apparently the first virus disease of bulbous plants to be fully described.

Acknowledgments.

The writer has to record his indebtedness to the Director and Board of Agriculture, Bermuda, for permission to publish this paper. He is also grateful to the Imperial Bureau of Entomology and Mr F. Laing and Mr F. V. Theobald for the identification of insects, and to Prof. H. H. Whetzel, of Cornell University, for suggestions.

LITERATURE CITED.

(1) BISHOP, GEORGE A. (1898). *A report on the diseases affecting the lily in Bermuda, their cause, prevention, etc.* Hamilton, Bermuda.

(2) GRIFFITHS, DAVID. (1924). The production of narcissus bulbs. *U.S. Dept. Agr. Bull.* No. 1270, Oct. 11, Washington, p. 24.

(3) —— (1922). The production of tulip bulbs. *Ibid.* No. 1082, Oct. 19, Washington, p. 37.

(4) KEAN, A. L. (1890). The lily disease in Bermuda. *Bot. Gaz.* XV, Jan., p. 8.

(5) KUNKEL, L. O. Further studies on the intracellular bodies associated with certain mosaic diseases. *Bull. Haw. Sugar Planters Assn. Bot. Series,* III, Pt. 2, p. 108.

(6) MCKINNEY, H. H. (1925). A mosaic disease of winter wheat and winter rye. *U.S. Dept. Agr. Bull.* No. 1361, Sept.

(7) OGILVIE, L. (1925). In *Report of the Board and Department of Agriculture, Bermuda.*

(8) —— (1927). An important virus disease of *Lilium longiflorum* and its varieties. *Nature,* CXIX, No. 2997, April 9th, p. 528.

(9) SOUTHWELL, H. (1926). The raising of hyacinths in Holland. *Journ. Min. Agr.* XXXIII, No. 3, June, p. 248.

(10) STOREY, H. H. and BOTTOMLEY, A. M. (1928). The rosette disease of peanuts (*Arachis hypogaea* L.). *Ann. Appl. Biol.* XV, No. 1, Feb., p. 26.

(11) WILSON, E. H. (1925). *The Lilies of Eastern Asia.* London, Dulau & Co.

(12) WOODS, ALBERT F. (1897). The Bermuda lily disease: a preliminary report of investigations. *U.S. Dept. Agr. Bull.* No. 14, Washington.

(13) —— (1902). The relation of nutrition to the health of plants. *Yearbook of the U.S. Dept. of Agr.,* Washington, p. 173.

(14) *Agr. Gaz. New South Wales* (1926), XXXVII, No. 8, pp. 603–612, No. 9, pp. 697–705.

(15) Bermuda—*Report of the Board of Agriculture,* 1898, pp. 1, 2.

(16) *Ibid.* 1900, p. 11.

(17) *Country Life in America,* April, 1904. (Quoted in *Royal Gazette,* Bermuda, 1904, p. 2.)

DESCRIPTION OF PLATES XXX—XXXIII.

Fig. 1. "Lilium Harrisii" (*L. longiflorum* var. *eximium* Baker).

Fig. 2. Yellow flat. On the left is a plant grown from a bulb which was produced by a plant some 2½ ft. in height, which showed current season infection in 1926, similar to that illustrated in Fig. 4. The normal plant to the right was produced by the smallest size of marketable bulb, 7–9 in. in circumference. March, 1927.

Fig. 3. Yellow flat. Grown from a bulb infected the previous year, the plant in 1926 being similar to that shown in Fig. 4. Figure enlarged. April 8th, 1927.

Fig. 4. Yellow flat. Current season infection by transfer of aphids. The top part of the plant is pale green in colour. *Aphis gossypii* may be seen on the under surfaces of the top leaves. February, 1928.

Fig. 5. Yellow flat. Copy of a photograph taken on April 2nd, 1915, by W. A. Orton in the greenhouses of the United States Department of Agriculture, Washington. The plants on the left are clearly from infected bulbs. The plants are from a group of bulbs said by Mr H. E. D. Smith to have been taken from plants affected with "yellow disease" in 1914.

Fig. 6. Yellow flat. Large patch of plants showing current season infection in the field. Normal plants in left foreground. Longbird Island. February 5th, 1925.

Fig. 7. Yellow flat. Current season infection by transfer of aphids. Note twisting of leaves and distorted buds and flowers. April 8th, 1927.

Fig. 8. Yellow flat. Three plants in pots (two of which are seen in the centre), badly infested with aphids, were placed amongst 11 healthy plants under a cheesecloth cage on January 15th, 1927. On February 1st symptoms were seen on the topmost leaves of all the plants. Photograph taken early in March. Normal plant on right.

Fig. 9. Yellow flat. Healthy plants infected by transfer of aphids under a cheesecloth cage on December 28th, 1927. Five of the plants show infection. The sixth showed the symptoms later. February 1st, 1928.

Fig. 10. Yellow flat. Patch in a field showing current season infection. Note the small plant from an infected bulb. March, 1928.

Fig. 11. Yellow flat. Badly diseased field. Note that the leaves of the diseased plants are dying off from the base upwards while those of the healthy plants remain green. May, 1925.

Fig. 12. Yellow flat. Current season infection on "Lilium giganteum." Normal plant on right.

Fig. 13. Yellow flat. In the background is a group of plants grown from bulbs of plants infected by transfer of aphids in December, 1926. Healthy plant of same age at left. In foreground plants infected in season 1925–26. February, 1928.

Fig. 14. Yellow flat. Plant infected in season 1925–26, photographed in February, 1928. It has completed its season's growth.

Fig. 15. Yellow flat. Left. Bulbs from plants infected in season 1925–26. Right. Bulbs 6½ in. in circumference when planted at beginning of season. The bulbs on the left would normally be as large if not larger than those on the right. This photograph indicates well the effect of the disease on yield. Dug July 27th, 1927.

Fig. 16. Yellow flat. Bulbs from plants infected in season 1925–26 and dug July 27th, 1927. Compare with Fig. 17.

Fig. 17. Yellow flat. The six bulbs were all the same size when planted in October, 1926. The plants from which the five smaller ones were gathered were infected with the disease by transference of aphids on January 26th, 1927. Note the compact centres of the infected bulbs, their small size, and their tendency to split.

Fig. 18. Yellow flat. Plants from scales of an infected bulb. Healthy plant on left. March, 1928.

(*Received March* 21*st*, 1928.)

OGILVIE.—A Transmissible Virus Disease of the Easter Lily (pp. 540–562).

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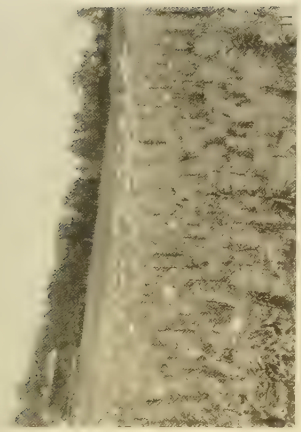
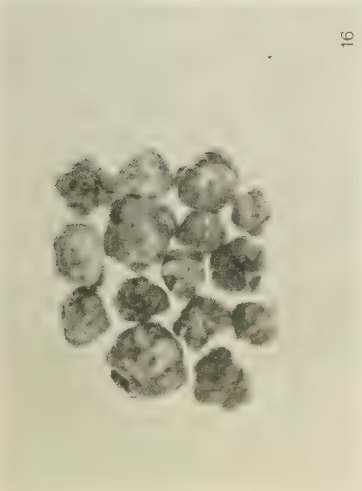
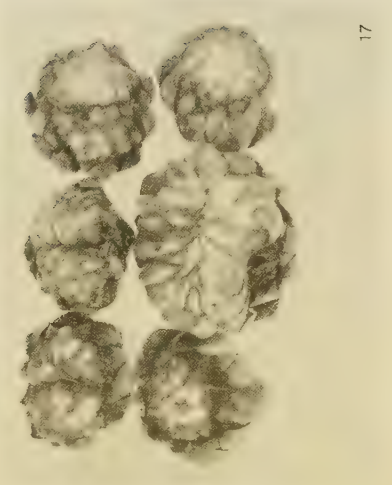


OGILVIE.—A Transmissible Virus Disease of the Easter Lily (pp. 540 562).





OGILVIE.—A Transmissible Virus Disease of the Easter Lily (pp. 540–562).

SPRAIN OR INTERNAL RUST SPOT OF POTATO

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(With Plates XXXIV–XXXVI and 7 Text-figures.)

Introduction.

The literature on this disease and on Net Necrosis, which has been associated with it, reveals a confusion, in part due to lack of definition of the diseases and in part to loose terminology. Horne (6, 7) distinguished two forms of brown discoloration in the flesh of the potato tuber which he called blotch or Internal disease and streak or Sprain, but mentions that the Board of Agriculture had adopted the name Sprain to include both blotch and streak. Similarly, Paine (12) distinguished between two types, A and B. He regarded Type A as that previously known as Sprain in which "the storage tissue of the tuber is spotted with islands of a reddish-brown colour," and Type B as identical with what is known as Net Necrosis in England and America. Net Necrosis is, however, variously described. Thus, W. A. Orton (9) says, "It is characterised by the occurrence of narrow streaks or dots of browned tissue outside of the vascular ring," but C. R. Orton (10) figures the same disease with the browned tissues mainly within the vascular cylinder, and draws no distinction between it and "Internal Brown Spot." Güssow (5) describes a disease under the same name in which the brownish internal discoloration travels along the vascular bundles of the tuber from the heel end towards the eye end. Schultz and Folsom (17) apparently regard Net Necrosis as identical with Phloem Necrosis and, as such, a symptom of Leaf Roll. Jones, Miller and Bailey (8) describe a necrosis of the internal phloem resulting from frost, and it is to this necrosis in particular that Paine (12) finds a parallel with his Type B of Internal Rust Spot. In Atanasoff's paper on Sprain or Internal Rust Spot (1) the description of the disease tallies closely with that of Horne (6) for "Internal Disease" and of Paine (12) for "Type A" Internal Rust Spot. No mention is made of any other type, and it is obvious that the author regards any spots or streaks which do not conform to this description as being of a different nature. In his paper on Net Necrosis (2) he uses this term to connote certain necrotic

lesions of the parenchyma and not of the vascular tissues, and is thereby at variance with Schultz and Folsom (17) and Gilbert (4) and others who have limited the term as before mentioned. A comparison of Atanasoff's photographs of Net Necrosis with those of Internal Rust Spot shows a striking similarity, and the author makes no definite statement as to how these diseases may clearly be differentiated. It is to be assumed that the presence of cork in the lesions of the latter and its absence in the former are the contrasting characters. He furthermore finds Net Necrosis in the tuber to be a symptom of Aucuba Mosaic and thus a hereditary complaint.

Lastly, Quanjer (15) states that "Net Necrosis" increases as a rule during winter, while with "Sprain" this is *nearly* not the case, but Atanasoff (1), speaking of Sprain, says "the number and extent of the rusty spots and of the browning usually increase considerably during the first months subsequent to the lifting of the potatoes," and again, that certain badly affected varieties "undergo a kind of dry rot during the winter, whereby the whole interior of the tuber becomes brown and perforated by numerous cavities." From the context, the inference would appear to be that this dry rot is extraneous to the disease itself and due to secondary organisms—a conclusion scarcely to be justified by the description.

From this summary it is apparent that with each new contribution to the subject the origin and nature of the rusty brown spots in the potato tuber have become still further complicated.

Misconceptions and contradictions of the kind noted would scarcely have arisen if figures illustrating the internal structure of the lesions described had been given. In this connection, it may be pointed out that with all the spots, streaks, arcs and blotches described in the literature as symptoms of Sprain, Internal Rust Spot, Brown Fleck or Internal Brown Spot, not a single drawing or microphotograph showing the minute structure of a section through the diseased areas is to be found, and with one exception (that of a micro-photograph by Atanasoff (2)) the same may be said of Net Necrosis.

Description of the disease.

In the virulent form of "Sprain" or "Internal Rust Spot" with which we have been dealing, there appeared from macroscopic observation to be two types of necrosis which may be described as follows:

(1) Rusty brown lesions in the form of isolated spots, streaks, or irregular blotches in which cavities are frequently present and which

often coalesce to produce large areas of disease. These lesions are found without and within the vascular cylinder, and the latter is sometimes crossed by them. The early stage of this disease agrees closely with that described by Horne(7) as Sprain, and by Paine(12) as Internal Rust Spot (Type A). The more virulent form with which neither Horne nor Paine appears to have been familiar is found by the author to be merely a later stage of the same disease and to be identical with the Sprain or Internal Rust Spot as described by Atanasoff(1) (see Plate XXXIV, fig. 1, and Plate XXXV, fig. 3). Sections through these lesions reveal a structure which is typical and diagnostic, whilst, except for certain variations due either to the tissue in which the lesions are formed or to the development of the disease, this structure is constant.

(2) Rusty brown discoloration of the vascular ring which appears in isolated spots or streaks, or which may form a complete ring. In the latter instance the disease bears a resemblance to Ring bacteriosis (*B. solanacearum*) from which it differs by the absence of any bacterial exudate, by the production of cork tissue surrounding the diseased elements and by the fact that it does not spread to the tissues on either side of the vascular ring (see Plate XXXV, fig. 2).

In the early stages of this work it was thought that these two types of brown discoloration were symptoms of the same disease. It was eventually found that that described under (2) was entirely distinct from Sprain and that it was a bacterial disease. For reasons which will appear later we suggest the name of "Corky Bacteriosis of the xylem" for it. This disease may occur in the tuber independently of Sprain but frequently accompanies it. It is by far the less important of the two diseases, and in the case under investigation accounted for not more than 2 per cent. of the tissue destruction. We should perhaps have been justified therefore in omitting Corky Bacteriosis from further consideration, and had we done so our work would have been greatly simplified. In the first place, however, it was felt that unless it was included, this particular investigation would have been less complete, and in the second place, this form of necrosis was of great interest from its superficial resemblance to the ring type of Frost necrosis recorded by Jones, Miller and Bailey(8) and to the tracheomycosis caused by *Verticillium albo-atrum* and *Fusarium oxysporum*.

TYPE OF SOIL IN WHICH THE DISEASES OCCUR.

The local soil on which the diseases occur has a very light sandy texture with the peculiarity that it is very dark in colour and resembles a peat. The drainage water is reddish brown, suggesting the presence of much iron. Analysis of the soil gave the following results:

Analyses of Soil Samples[1].

	Sample A	Sample B
Lime requirement	Nil	Nil
Pot. thiocyanate test	No colour	No colour
Pot. salicylate test	,,	,,
*p*H (colorimetric method)	6·6	6·7–6·8
Mechanical Analysis.		
	%	%
Fine gravel	0·60	0·56
Coarse sand	59·18	52·25
Fine sand	23·68	28·21
Silt	2·60	2·93
Fine silt (*a*)	2·75	3·89
Fine silt (*b*)	2·95	2·38
Clay	1·10	1·00
Moisture	1·58	1·75
Loss on ignition	3·58	4·41
*Loss on solution (*N*/5 HCl)	2·99	3·86
	101·01	101·24
CO_2	0·510	0·562
*containing $CaCO_3$	0·80	1·01
*containing $MgCO_3$	0·34	0·25

The loss on ignition includes the CO_2 so that the organic matter in each sample is 3·07% and 3·85% respectively.

The outstanding feature of these analyses is the extremely low figures for the organic matter content of the soils, and from a practical standpoint these are confirmed by the fact that the land requires heavy dressings of manure.

Quanjer (15) states that Sprain "is common in soils where, on account of the low amount of lime, Actinomyces—scab is absent," but in this instance the reverse statement might be made, since the soil in question is one of the worst scabbing soils we know. That it is not deficient in lime is shown by the analyses. The diseases in question were indeed so often associated with Common Scab that we were led to surmise a common

[1] These analyses were kindly carried out by our colleague Mr H. Trefor Jones, M.Sc., Assistant Lecturer in Agricultural Chemistry, and Advisory Chemist, University of Leeds.

causal factor for their occurrence, and on this account a test of the action of green manure as a means of control was included in our field experiments.

ISOLATION OF CAUSATIVE ORGANISMS.

The material first selected for this purpose consisted of the Sprain or Internal Rust Spot lesions. Microscopic examination showed that the larger blotches in which cavities had formed were often inhabited by fungal threads and bacteria. *Rhizoctonia solani* was easily isolated from such areas of disease and pure cultures of this fungus were made for further use. The smaller spots were free from fungi, and although some of the cells within the spot sometimes showed numbers of minute particles in motion, it was not found possible to demonstrate these to be bacteria. Spots of disease not exceeding 3 mm. in diameter and situated entirely in the cortex or pith were chosen as inoculum material. Observing the usual precautions of cultural technique, attempts were made to obtain cultures from such spots on the ordinary nutrient media and on a great variety of specialised media but with no success. A medium was then prepared according to Cunningham(3) from an extract of the soil in which the disease occurred. This was sterilised by steaming on three consecutive days, and when thus prepared gave an opalescent liquid with a brownish tinge and a reaction (pH 6·9)[1].

Twelve tubes of this medium were inoculated each with a typical diseased spot and incubated at 25° C. After 4 days, three of the tubes showed a faint opacity of the medium, and in each it was found possible to demonstrate the presence of bacteria by means of stained slides. On plates poured with Soil extract agar minute colonies were seen under the microscope in 4 days and became visible to the naked eye after 10 days. Pure cultures were made and labelled S_1.

Attention was next directed to the lesions of Corky Bacteriosis. Here out of six tubes of soil extract inoculated three developed an opacity after 5 days' incubation. The colonies subsequently given on Soil extract agar and the slope cultures on the same media were so similar to those of S_1, that the cultures were at first thought to be identical. Subsequent work established great differences. The new culture was labelled CB_2.

[1] If the medium was autoclaved instead of being steamed a precipitate was formed and the medium was not so satisfactory.

Morphological and cultural characters of the organisms S_1 and CB_2.

After a number of sub-cultures in soil extract had been made, both organisms showed a capacity for growth in potato broth, nutrient gelatine and in various sugars. A very weak growth of S_1 was also obtained on nutrient potato agar but, with this exception and that of soil extract agar, neither organism could be grown on agar media. The characters of the two organisms are given in tabular form below:

	S_1	CB_2
Form	Short rods frequently in pairs	Thin rods sometimes in pairs
Average length	1·6 μ	2·6 μ
Average breadth	0·5 μ	0·6 μ
Motility	Very motile	Motile, but less so than S_1
Gram stain	Negative	Negative
Flagella	Not demonstrated	Not demonstrated
Spore formation	Nil	Nil
Colonies on soil extract agar plates (pH = 7)	Visible to naked eye after 10 days, becoming 1–1·5 mm. in diam.	Same appearance as S_1
	Round to irregular, dewdrop in appearance, later opalescent; faint steely blue	
Soil extract agar slope (pH = 7)	Very slight growth after 4 days—seen only with difficulty. Dewdrop in appearance. Individual colonies very minute	Very slight growth in 5 days after heavy inoculation Same appearance as S_1
Soil extract solution (pH = 7)	Slight cloudiness after 3 increasing up to 7 days' growth remaining in suspension	Same appearance at first as S_1, but giving after 6 days flaky mass at bottom of tube with slight cloudiness in medium
Potato broth (pH = 7)	Good growth after 3 days with cloudiness and flocculent mass at bottom of tube	Same appearance as S_1, but later producing much greater deposit which differs from S_1 in being mucilaginous
Colonies on nutrient gelatine plates (pH = 7)	Visible under microscope after 4 days and never becoming greater than 1 mm. in diam. Round to irregular. Granular. "Primuline yellow" (Ridgway (16)). Liquefaction slow, saucer-shaped, colonies remaining intact	Visible under microscope after 4 days and not more than pin point after 16 days. Dewdrop in appearance. Under magnification the colonies are granular and the margins entire to slightly undulate. Liquefaction nil
Nutrient gelatine stab*	Upper portions filiform	Stab as for S_1
	Lower portions beaded	Liquefaction nil
	Liquefaction started after 7 days, at first saccate, later infundibuliform	Surface growth. "Cream buff" (Ridgway (16))

* 15% gelatine (pH = 7). Culture incubated at 23·5° C.

	S_1		CB_2	
	Surface growth good "Primuline yellow" (Ridgway (16))			
Potato plug	No growth		Poor growth, putty coloured	
Sugar reactions*		After days		After days
Lactose	+	30	K	16
Glucose	K	8 (becoming neutral again after 16 days)	K	,,
Saccharose	K	16 (becoming acid after 31 days)	K	,,
Mannite	K	8	K	,,
Laevulose	.	45	K	,,
Maltose	.	45	K (slight)	45
Arabinose	+	30	K	30
Galactose	+	30	K	16
Sorbite	K	36	K	16
Dextrin	K	16	K	16
Inulin	.	45	.	45
Dulcite	.	45	.	45
Salicin	.	45	.	45
Adonite	.	45	.	45
Glycerine	.	45	.	45
Litmus milk	.	45	K	45
Potato broth	+	30	K	16
Potato starch	K	16	K	16

+ = acid without gas. K = alkalinity.

* Including also reactions obtained in litmus milk, potato broth, and potato starch. Except for litmus milk the indicator used was brom-cresol purple and the initial reaction of the media was pH = 6·8.

LIMITS OF HYDROGEN ION CONCENTRATION OF THE MEDIA WITHIN WHICH THE ORGANISMS GROW.

This was tested by stab cultures in nutrient gelatine with the following results:

Initial pH of medium	S_1	CB_2
5·7	Fair growth along upper part of stab	No growth
6·0	Fair growth along whole line of stab	Fair growth
6·3	,, ,, ,,	,,
7·4	,, ,, ,,	,,
7·7	,, ,, ,,	,,
8·0	No growth	,,

On potato broth S_1 was found to give slight growth at pH = 5·3.

Changes in hydrogen ion concentration produced by growth of the organisms in potato broth.

The final estimations of the *p*H value of the media were made after 41 days. It was then found that in the case of organism CB_2 the *p*H value was, in every case but one, greater than 8·4, and since the best indicator available was phenol red the increasing depth of colour outside its range was indicated by plus signs.

Initial *p*H of medium	S_1 *p*H after 41 days	CB_2 *p*H after 41 days
7·4	6·9–7·0	8·4+
7·3	6·8	8·4+
7·2	6·6–6·7	8·4++
7·1	6·6–6·7	8·4++
7·0	—	8·4++++
6·9	6·5	8·4++
6·8	6·4–6·5	8·4++
6·7	6·4–6·5	8·4+
6·5	6·4–6·5	8·4+
6·4	6·3–6·4	7·8
6·3	6·2	8·4+
6·2	6·2–6·3	8·4+

It will be obvious from the tables given that the two organisms differ widely in their cultural characters, and that, moreover, neither bears any resemblance to the Pseudomonas solaniolens described by Paine (12). Both appear to be new species. It is proposed to name S_1 the organism of Sprain, *Bacterium rubefaciens*, and CB_2 the organism of Corky Bacteriosis, *Bacterium suberfaciens*.

Inoculation experiments.

Experiment I—1926. At the time of these experiments it was uncertain whether or not the symptoms of Sprain and Corky Bacteriosis were phases of one disease and consequently, whether or not the two organisms which had been isolated acted independently or in combination. Moreover, the disease, as we knew it, was apparently much more virulent than that described as Sprain by previous workers, and it was thought that this might possibly be accounted for by the aid of yet another organism, namely, *Rhizoctonia solani*—so frequently found in the lesion cavities. A series of inoculation experiments were therefore carried out as shown in the following table:

No. of series	Soil inoculated with cultures of:
(1)	S_1
(2)	CB_2
(3)	S_1 and CB_2
(4)	S_1 and *Rhizoctonia solani*
(5)	CB_2 and *Rhizoctonia solani*
(6)	$S_1 + CB_2$ + *Rhizoctonia solani*
(7)	Untreated

The sets were sterilised by immersion in 0·1 per cent. solution of mercuric chloride for $1\frac{1}{2}$ hours and sprouted under as aseptic conditions as possible.

Owing to lack of greenhouse accommodation the experiment was carried out in the open, the pots being placed on raised forms standing on short grass. The inoculations of S_1 and CB_2 were made by pouring potato broth cultures of these organisms into the pots, whilst in the case of *Rhizoctonia solani* emulsions of the fungus grown on nutrient potato agar were added.

The first inocula were stirred into the soil before the potatoes were planted, and subsequent inocula were added at intervals of one month during May, June and July. Even so, the total inoculation of S_1 and CB_2 was on the weak side for soil impregnation since heavy cultures of these organisms could not be grown. The plants were healthy and vigorous and the resulting crops were apparently free from extraneous diseases. The progeny of each plant was harvested and stored in a separate bag until February 1927 when the tubers were cut into thin slices and examined.

Apart from any disease, many tubers—especially of certain varieties—show a browning of the vascular ring at the extreme heel end, due probably to the natural rotting away of the stolon. Hence, on examining the tubers no notice was taken of any tissue browning, unless it appeared below a depth of $\frac{1}{4}$ in. from the proximal end of the tuber.

The results of infection are given in the following table (p. 572).

The type of infection occurring in the Series 1 and 4 (*i.e.* resulting from inoculation with S_1) was obviously different even to the naked eye from that shown in the Series 2 and 5 (resulting from inoculation with CB_2). A photograph of a cut tuber from the former series is shown in Plate XXXVI, fig. 6, where the lesions consist of spots in the cortex or pith. A large number of such spots or blotches were sectioned and were found to correspond exactly with the descriptions of Internal Rust Spot as given by Paine (11). The spots varied from 1 to 6 mm. in diameter, and in most of the infected tubers several occurred. These were distri-

Series	No. of pot	Total No. of tubers in crop	No. of tubers showing infection
1	1	7	5
	2	9	3
	3	8	5
2	4	8	2
	5	8	4
	6	7	3
3	7	10	4
	8	9	2
	9	8	5
4	10	6	4
	11	7	3
	12	8	2
5	13	14	3
	14	10	1
	15	8	3
6	16	8	5
	17	10	5
	18	6	1
7	(control) 19	12	0
	20	10	0
	21	10	0

buted in the flesh of the tubers and did not preponderate either in the heel or rose end of the potato.

The type of infection produced by organism CB_2 and found in the Series 2 and 5 agreed in every respect with that which has been described as Corky Bacteriosis. In the Series 3 and 6 where a combined inoculation of the two organisms was made Corky Bacteriosis only was reproduced. We must infer that the cultures of S_1 were in some way inactivated by those of CB_2 and suggest that this may have been brought about by the strongly alkaline nature of the CB_2 potato broth culture which was poured over the soil at the same time as that of S_1. A photograph of the inoculation results is shown in Plate XXXVI, fig. 9.

Rhizoctonia solani in Series 4 to 6 produced numbers of typical black sclerotia on the tubers, but its presence did not affect the internal lesions in any way. Moreover, no trace of fungal threads was found in any of the spots. It seems probable therefore that this fungus along with other secondary organisms finds its way into rust spots only after the tubers have become honeycombed with disease and a natural means of entry thereby provided by shrinkage and cracking of the tissues.

So far as the above description goes it would appear that the problem of the causal organisms of both diseases had been solved, but a serious obstacle to this conclusion appeared.

Whilst in tubers from Series 1 and 4, the sections of the spots in the

cortex and pith entirely confirmed the diagnosis of Sprain, some very small brown streaks also appeared in the vascular ring of the same tubers and were found to consist of brown lignified wood vessels. A detailed description of the morphology of these lesions is given later, and from this it will appear that this tracheomycosis differed from that of Corky Bacteriosis in that it was not accompanied by cork formation. At the time, however, we thought that there might be a connection between the two, and it was feared that some cross infection might have occurred between the pots especially as these had stood in the open during the whole season. For this reason the experiments were repeated the following year.

Experiment II—1927. The use of a greenhouse had now been secured and the experiment could thus be carried out under more stringent conditions. Seed tubers of an early variety (Sharpe's Express) and a late variety (Field Marshal) were obtained. After sterilisation, four of the tubers were cut into halves and the two halves of each were placed in a sterile covered dish with a piece of sterile and moistened filter paper. The dish was then kept in the dark for 48 hours. In this way, as shown by Priestley and Woffenden (14), a firm healthy cork layer was formed over the cut surfaces of the tubers.

Four of the half tubers were used for inoculation and the other four half tubers served as controls. *Rhizoctonia solani* was not included in the inoculations, and in Series 3, where a combined inoculation of S_1 and CB_2 was made, a period of a week was allowed to elapse between the two inoculations. In other respects the experiment was carried out as in 1926. The series of inoculations with four plants in each were arranged as follows:

Series	No. of pot	Variety	Inoculum added to the soil
0	1	Sharpe's Express	Control plant
	2	,,	,,
	3	Field Marshal	,,
	4	,,	,,
1	5	Sharpe's Express	S_1
	6	,,	,,
	7	Field Marshal	,,
	8	,,	,,
2	9	Sharpe's Express	CB_2
	10	,,	,,
	11	Field Marshal	,,
	12	,,	,,
3	13	Sharpe's Express	$S_1 + CB_2$
	14	,,	,,
	15	Field Marshal	,,
	16	,,	,,

The crops were lifted in October. Three tubers from each pot were then cut, and of these four tubers in Series 1 showed small but typical Sprain spots, whilst five in Series 3 showed a mixed infection of Sprain and Corky Bacteriosis. The remaining tubers were stored and finally examined on January 10th, 1928. Every spot in the parenchymatous tissues or spot or streak in the vascular ring was sectioned in order to determine its type. The results are given in the following table:

Series	Inoculum	Total no. of tubers	No. of tubers infected	No. and type of spots or streaks		
				Sprain	Tracheomycosis without cork formation	Corky Bacteriosis
0	Control	26	0	0	0	0
1	S_1	23	10	11	2	0
2	CB_2	30	4	0	0	4
3	S_1+CB_2	25	10	17	2	2

In the case of the organism CB_2 the infection was less than that obtained in Experiment I, and this was probably due to the high temperature of the greenhouse which could not easily be reduced and often went to 37° C. In artificial culture both organisms were found to grow best at a temperature of 20–25° C. and no growth was obtained at 37·5° C. This result, however, was not of much consequence since the pathogenicity of the Corky Bacteriosis organism was not in question. The browning of the vascular ring which did occur was very typical.

The results were more interesting in respect of the organism S_1. Here, both in the Series 1 and 3, the spots produced were of the typical Rust Spot type (see Plate XXXVI, fig. 7). On the other hand, the tracheomycosis which was observed in the 1926 experiment was again present in two of the infected tubers of Series 1 and two of those in Series 3.

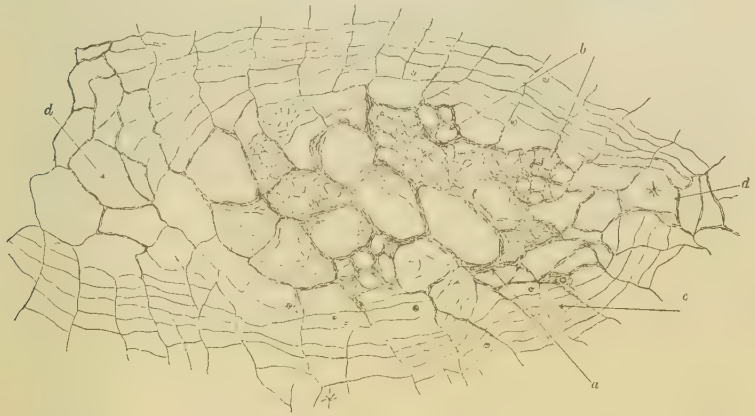
In these four tubers it was precisely similar to that previously seen, consisting of thickened lignified brown xylem vessels unaccompanied by other evidences of disease or by cork.

From the point of view of tissue destruction this phenomenon is insignificant, but whether it is peculiarly associated with Sprain or whether, as we have some reasons to suspect, it also accompanies other tuber diseases, we think that it should be recorded.

HISTOLOGY OF THE LESIONS.

Sprain or Internal Rust Spot.

Text-fig. 1 shows a section of a small spot of the disease taken from the cortex of a naturally infected tuber, and in Plate XXXVI, fig. 8, a micro-photograph is given of a section of a slightly larger spot (also in the cortex) produced by inoculation with S_1. The structure is identical in both cases. The spot consists of a group of parenchymatous cells the walls of which are brown, thickened and lignified. Some of these cells lose their starch contents, but in young spots of the type figured there are invariably cells which are still packed with starch grains. In such



Text-fig. 1. Section through a small spot of Sprain in the cortex of a naturally infected tuber showing some empty cells and other cells still filled with starch grains. (*a*) thickened suberised and lignified cell walls, (*b*) starch grains in cells, (*c*) cork, (*d*) points at which the disease has spread outwards before the cork layer was complete.

cells the spaces around the starch grains are filled with minute, granular bodies which readily stain with Methylene Blue and other proteid stains. When Rust Spots occur in the cortex a cork meristem arises in the surrounding parenchyma at an early stage and usually at a distance of two or three cells from the seat of the disease. It is always well marked and the resulting cork varies in thickness from four to ten cells. When this corky layer completely surrounds the diseased area it is probable that there is no further development of the spot, but it frequently happens that at one point or another a gap occurs in the cork band. Here,

apparently either cork formation has lagged behind the disease or as has sometimes been seen, the cells cut off by the cork meristem have been themselves infected by the disease before they were suberised. In this way the disease spreads and irregular spots or streaks are formed.

In sections stained with Sudan III the affected parenchymatous cells and also the inner layers of the cork band readily took up the stain, whilst the walls of the outermost layer of cork were not stained. It was clear therefore that the interior of the spot was highly suberised and that the meristem of the cork lay in the outer row of the cork band.

Text-fig. 2. Section through part of a large Sprain spot showing cavities in the pith of a naturally infected tuber. (*a*) debris of diseased cells which have collapsed, (*b*) parenchyma cells some of which contain starch grains (not shown), (*c*) cork layer which is not so regular or well developed as in the cortex.

Sections stained with safranin and light green showed good differentiation. The walls of the affected parenchyma cells within the spot and frequently those of the first one or two layers of cork adjacent to it stained a deep red, whilst the outer layers of the cork and the healthy tissue beyond it took up the green stain. The affected parenchyma was thus shown to be lignified as also were sometimes the walls of the inner cork layers. It should be pointed out that the brown colour of the parenchymatous cell walls often masks the Sudan III and safranin stains, and these are best seen in the less strongly affected cells.

In the pith of the tuber the development of the Rust spots is slightly different. Here, particularly in the regions where the cells are less closely packed with starch, the cork meristem arises at a greater distance from the diseased cells than in the cortex, and its development is slower and poorer. It is frequently not more than 3 to 4 cells thick and is generally less suberised than in the cortical Rust spots. This difference in the production of wound cork undoubtedly accounts for the greater development of the lesions of Sprain and consequent tissue destruction in the pith than in the outer cortical tissues of the potato.

An illustration of such a spot in the pith of the tuber is given in Text-fig. 2, and here also a cavity is shown. These cavities occur in all old Rust spots but are more frequently seen in those of the pith. They are due to the disruption of the lignified cells within the spot.

Corky Bacteriosis

The illustration given (Text-fig. 3) is that of a primary bundle from a naturally infected tuber in which the spots of disease were sufficiently numerous to give the appearance of a continuous brown ring. Actually,

Text-fig. 3. Section through vascular ring of a tuber showing Corky Bacteriosis of the xylem: (*a*) brown necrotic vessels of the primary xylem, (*b*) cork layer which closely invests the affected tissues.

in this stage the whole of the xylem is not affected and some still healthy xylem bundles alternate with the diseased ones.

To the naked eye these spots, and later the whole discoloured ring, are a deeper brown than the spots of Sprain, and this deeper colour is even more obvious under the microscope. The only tissue affected is the xylem, in which the walls of the vessels are considerably thickened and distorted. The vessels are generally empty but are sometimes filled with a granular brown mass. Where the wood is primary, a layer of cork from 4 to 15 cells thick generally completely surrounds it.

Marked differences were found between this cork and that enclosing the spots in Sprain. First, it arises in those parenchymatous cells closely abutting on the affected xylem vessels and not, as in Sprain, in a layer two or more cells distant from the initial disease. Secondly, sections of diseased spots stained with Sudan III show that practically all the cork cells are heavily suberised and not, as in Sprain, the inner layers only. So quickly indeed does suberisation take place that it has been difficult to locate the cork meristem. Ultimately, however, sections were made in which the cells of the outer layer were found to be densely packed with protoplasm, whilst their walls were only slightly stained with Sudan III. In the same sections the cells of the inner layers were clear and their walls stained a deep red. The outer layer was therefore taken to be the meristem which is thus similarly placed in Corky Bacteriosis as in Sprain.

Where the disease was found attacking the primary bundles as figured, neither the Phloem bundles nor the adjacent parenchyma were affected, but where also secondary thickening had occurred, the tissues mentioned were often somewhat crushed. Here, the cork may form on the inner side of two or more of the xylem groups and may indeed cross the interfascicular cambium ring but rarely surrounds the xylem bundles. In a few very severe cases of Corky Bacteriosis, where the necrosis of the tissues had extended to several cells of the pericycle, one or two phloem groups were either engulfed or at least showed slight browning of the cell walls. Such cases are rare, and Corky Bacteriosis is essentially a disease of the xylem vessels. The spots produced by artificial inoculation were identical with those of the natural disease here described and additional figures have not therefore been given.

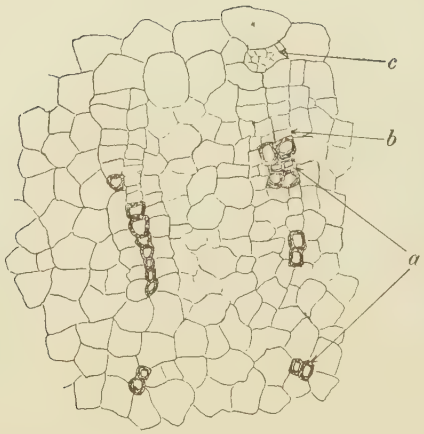
Tracheomycosis accompanying Sprain.

The illustration (Text-fig. 4) shows a section through a minute spot in the vascular ring of a tuber which had been inoculated with S_1 and showed several true Rust spots. Here, as in Corky Bacteriosis, the xylem vessels were a deep brown, but the walls were not so thickened and distorted as in that disease. In inoculation experiments these spots were very inconspicuous, few in number, and rarely exceeded 5 mm. in length. In tubers naturally infected with Sprain longer streaks have been observed, and these may occur in the secondary as well as in the primary xylem. Where it is found in vessels of the secondary xylem, two or three affected vessels frequently alternate with apparently healthy ones within the same bundle and are not necessarily found in a group as in Corky Bacteriosis (see Text-fig. 5). The main difference between this phenomenon and the disease of Corky Bacteriosis lies, however, in the fact that whereas, in the latter, cork formation is rapid and abundant, in the former, no cork is formed at all. This distinction is obviously of crucial importance and we have therefore been careful to verify it repeatedly. Moreover, in the Tracheomycosis associated with Sprain no tissues other than the xylem are at any time affected. It must not be thought that there is any likelihood of confusion between this Tracheomycosis and Corky Bacteriosis even when affected potatoes are examined by the naked eye, since the browning which appears from the former is slight compared with that produced by the latter. In fact, Sprain Tracheomycosis often only becomes apparent when sections are examined microscopically[1].

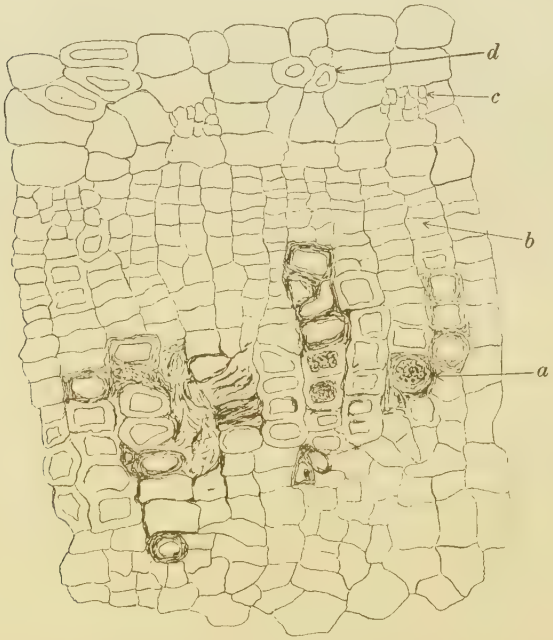
Entry of Sprain in the tuber.

Atanasoff (1) states that this disease obtains entry through the skin of the tuber where it produces "distinct scabbing of the periderm and slight or pronounced depressions in, and deformations of, the tuber." We have found little confirmation of these statements. One or two spots of Sprain were certainly found near the surface of the tuber, and at these points the skin of the tuber immediately above the spot was wrinkled and peeling. A section of such a spot is shown in Text-fig. 6 and from this it might be inferred that the organism has entered the tuber directly through the periderm. On the other hand, it is equally feasible to suppose

[1] A preliminary communication of this investigation by my colleague Dr W. A. Millard was published in *Nature* of December 4th, 1926, but at this time the morphological work done was not sufficient to enable us to draw a clear distinction between Corky Bacteriosis and the Tracheomycosis accompanying Sprain.



Text-fig. 4. Section through minute streak in the vascular ring of a tuber artificially infected with Sprain and showing Tracheomycosis unaccompanied by cork. (*a*) lignified xylem vessels, (*b*) cambium of vascular bundle, (*c*) phloem island.



Text-fig. 5. Section through a larger streak in the vascular ring of a tuber naturally infected with Sprain and showing extensive Tracheomycosis. (*a*) Necrotic xylem vessels, (*b*) cambium of the vascular ring, (*c*) phloem island, (*d*) bast fibres.

that the disease has reached the surface of the tuber from within and that the shrivelling of the skin overlying the spot is due to the pressure of the wound cork below the disease in the cortex.

Moreover, a very noticeable feature of Sprain disease is the occurrence of spots in the flesh of a tuber where no trace of any connection with the tuber surface can be found. Again, the Rust spots arise and develop

Text-fig. 6. Section through a spot of pinhead size on the surface of a "sprained" tuber where the skin was slightly shrivelled. At the point *X* a large and typical Sprain spot was present. (*a*) diseased parenchymatous cells, (*b*) periderm of the tuber, (*c*) new cork layer arising below the infected cells.

mainly during storage. It seems very possible, therefore, that the organisms enter through the lenticels of the young tubers or even through the stomata of the stolons before tuberisation and that they become distributed throughout the watery sap of the intercellular spaces of the tuber. As the tuber ripens the nature of the food supply and the increasing aeration provide conditions for further development of the organisms, and disease spots then arise indiscriminately in the tissues.

In certain varieties of potatoes, more particularly Golden Wonder, badly affected tubers are often misshapen, but this malformation is not a constant feature of the disease.

Origin and transmission of Sprain and Bacteriosis.

Two experiments will first be described, one, in which clean tubers were planted in infected soil, and the other, in which infected tubers from a farm crop were planted in sterilised soil.

Experiment I. Two tubers were taken from the supply of healthy Presidents already mentioned, and when cut showed no signs of any brown spots or streaks. After the cut surfaces had healed, the half tubers were sterilised in 0·1 per cent. corrosive sublimate for 2 hours, and

Text-fig. 7. Section through young shoot of a tuber infected with Sprain showing a typical spot of the disease in the parenchyma.

one-half of each tuber was planted in a pot of soil from the field in which the disease occurred. The progeny of each plant was badly affected with Sprain (see Plate XXXV, fig. 4). There is thus no doubt that this disease is conveyed to the crop from infected soil. No signs of Corky Bacteriosis were seen in the crop, but this negative result can scarcely be regarded as conclusive.

Experiment II. Two badly diseased tubers were sterilised in 0·1 per cent. corrosive sublimate for 2 hours and then planted in pots of

sterilised soil. The progeny of each plant showed marked infection with Corky Bacteriosis (see Plate XXXV, fig. 5), but none was infected with Sprain. It would appear, therefore, that Corky Bacteriosis is transmitted from the mother tuber to its progeny through the stolons, but that Sprain is not so transmitted.

The result in respect of Sprain agrees with the conclusions arrived at by Pethybridge(13) and Atanasoff(1) and is confirmatory of statements made by farmers that infected seed may be used with safety on heavy land. On the other hand, there is distinct evidence to the contrary. Thus, some diseased potatoes were allowed to tuberise in the laboratory where some of the new tubers arose directly on the mother tubers without the intervention of a stolon. In one of these tubers two spots of Sprain were found. Again, some diseased tubers were allowed to sprout in the laboratory and in one of the sprouts so obtained a typical spot of Sprain was found in the pith (Text-fig. 7). These results led us to plant fifty tubers of Golden Wonder from a badly sprained crop in a plot at Garforth, where Sprain had not been observed in previous potato crops. The crop obtained was for all practical purposes a clean one, but when after 4 months' storage one hundred tubers were carefully sliced, one or two specks of Sprain were found in fifty-eight of them. It would appear, therefore, that Sprain is transmissible from the mother tuber to the new crop, but that the degree of disease produced by this mode of infection is slight.

Summary.

1. In the present investigation necrosis of the tubers was found to be due to two distinct diseases: (1) Sprain or Internal Rust Spot, and (2) a disease hitherto unrecorded, which has been named Corky Bacteriosis of the xylem. The former is much the more important and accounts for 98 per cent. of the tissue destruction.

2. In Sprain the tissue attacked is the parenchyma both within and without the vascular ring. The lesions vary greatly in form and may consist of spots, arcs, streaks or irregular blotches. The larger lesions are generally hollow in the centre.

3. The histological structure of the lesions of Sprain varies slightly according to whether these occur in the starch-packed cortical tissue or in the pith where the cells are less densely filled with starch, but in either case, is characteristic. A feature of the spots is that they are more or less completely invested with a zone of cork the inner layers of which are suberised.

4. The soil on which Sprain occurs in a virulent form is a light sandy loam deficient in organic matter and one on which potatoes scab severely.

5. The disease is apparently slight when the crop is lifted but develops rapidly and continuously during storage.

6. Infection of the crop arises mainly from contaminated soil and not from affected seed.

7. The causative organism of Sprain has been isolated and typical spots of the disease have been reproduced by inoculation with it. It consists of a very short Bacterium, which, being new to the literature, has been named *Bacterium rubefaciens*.

8. There is little evidence that this organism enters the fully formed tuber through its skin, and isolated spots which have no apparent connection with the periderm are a feature of the disease. It is suggested that the organisms enter at a very early stage through the stomata or lenticels of the tuber-bearing stolon, remaining quiescent in the water-filled intercellular spaces of the tuber and becoming active only when the latter ripens.

9. In potatoes affected with Sprain a certain necrosis of the xylem vessels may occur but may easily be overlooked. This Tracheomycosis is essentially different from that of Corky Bacteriosis.

10. Corky Bacteriosis produces a browning and lignification of the vascular ring macroscopically resembling the Ring Bacteriosis of *B. solanacearum*.

11. It is essentially a disease of the xylem vessels which thereby become closely invested with a thick layer of suberised cork.

12. Corky Bacteriosis is transmitted from the mother tuber to its progeny by way of the stolons. It is also contracted from infected soil apparently by infection of the stolon or the stolon end of the tuber.

13. The causative organism of this disease has been isolated and the disease reproduced by inoculation. The organism being new to the literature has been named *B. suberfaciens*.

The writer wishes to express his great indebtedness to his colleague Mr W. A. Millard, D.Sc., for the invaluable help and guidance which he has so generously given him during the course of this investigation. His best thanks are also due to Mr J. Manby for the photographs.

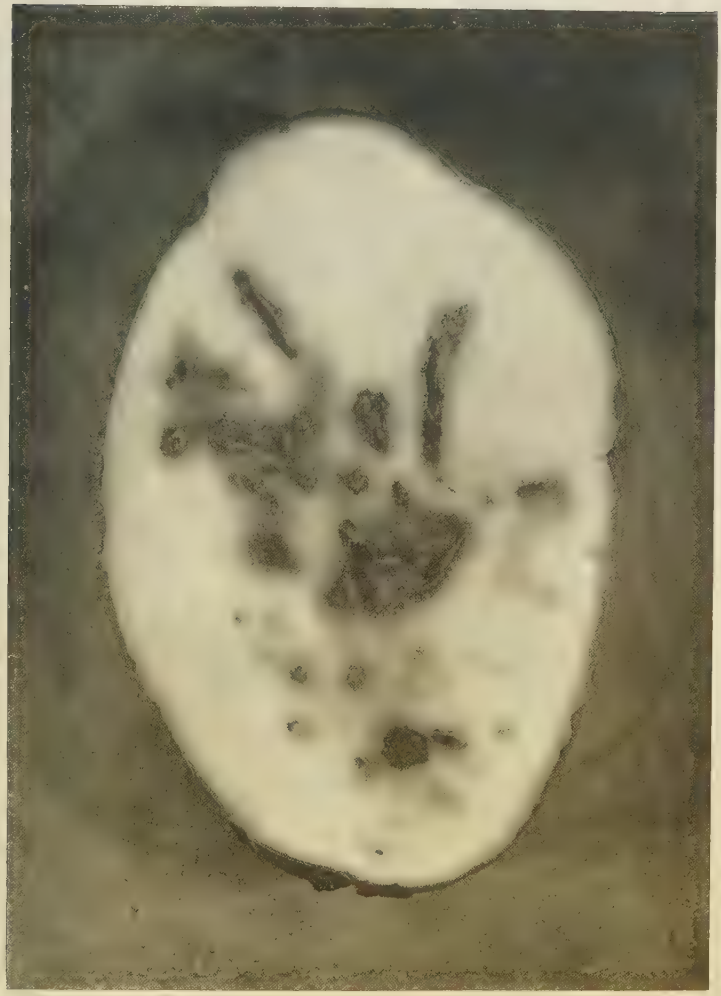


Fig. 1.

BURR.—Sprain or Internal Rust Spot of Potato (pp. 563–585).

Fig. 2.

Fig. 3.

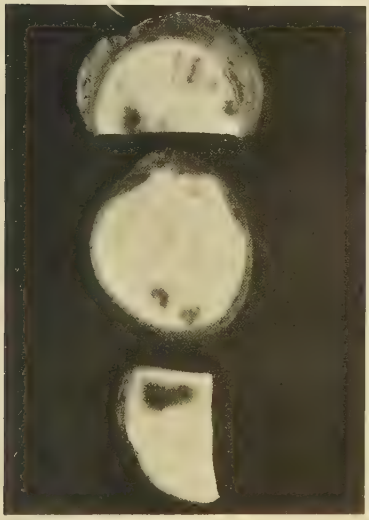


Fig. 4.

Fig. 5.

BURR.—Sprain or Internal Rust Spot of Potato (pp. 563–585).

Fig. 6.

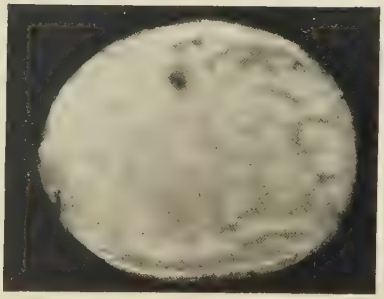


Fig. 7.

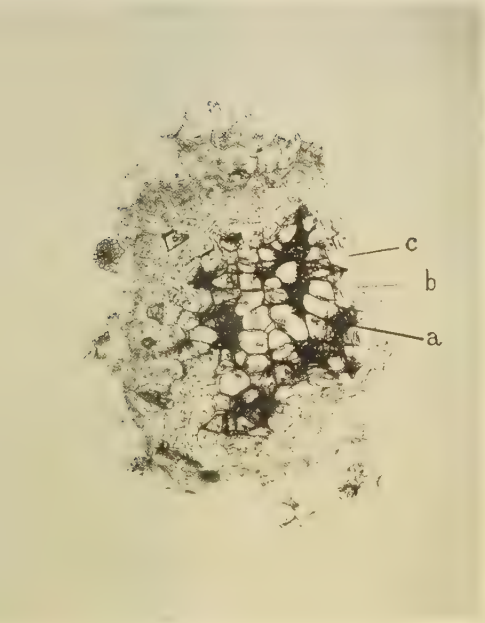


Fig. 8.

Fig. 9.

BURR.—Sprain or Internal Rust Spot of Potato (pp. 563–585).

LITERATURE CITED.

(1) ATANASOFF, D. (1926). *Phytopath.* XVI, 711–22.
(2) —— *Ibid.* XVI, 929–40.
(3) CUNNINGHAM, A. (1924). (Text-book.) Practical Bacteriology for students of Agriculture.
(4) GILBERT, A. H. (1927). *Phytopath.* XVII, No. 8, 555–61.
(5) GÜSSOW, H. T. (1915). *Rep. Div. of Botany,* II, Sessional paper 16.
(6) HORNE, A. S. (1910). *Journ. Agr. Sci.* III, 322–32.
(7) —— (1914). *Ann. App. Biol.* I, 183–203.
(8) JONES, MILLER and BAILEY (1919). *Agr. Exp. Sta. Univ. of Wis. Bull.* XLVI.
(9) ORTON, W. A. (1914). *U.S. Dept. Agr. Bull.* LXIV.
(10) ORTON, C. R. (1916). *Agr. Exp. Sta. Penn. Ste Coll. Bull.* CXL.
(11) PAINE, S. G. (1918). *Ann. App. Biol.* V, 77–9.
(12) —— (1923). *Rept. Internat. Conf. Phytopath. and Econ. Ent. Holland,* 1923, 74–8.
(13) PETHYBRIDGE, G. H. (1913). *Journ. Dept. Agr. and Tech. Instruct. for Ireland,* XIII, No. 3, 468.
(14) PRIESTLEY and WOFFENDEN (1923). *Ann. App. Biol.* X, 96–115.
(15) QUANJER, H. M. (1926). *Tijdschrift over Plantenziekten,* 32 ste Jg, April, 1926.
(16) RIDGWAY, R. (1912). *Color Standards and Color nomenclature.*
(17) SCHULTZ and FOLSOM (1921). *Journ. Agr. Res.* XXI, No. 1, 47–80.

EXPLANATION OF PLATES XXXIV—XXXVI.

Fig. 1. A naturally infected tuber after four months' storage showing the irregular spots and streaks of Sprain. A small spot of Corky Bacteriosis of the xylem is seen in the vascular ring at the base of the tuber.

Fig. 2. A naturally infected tuber after four months' storage showing Corky Bacteriosis of the xylem. The disease has apparently entered the tuber from the stolon.

Fig. 3. An earlier stage of Sprain than that of Fig. 1.

Fig. 4. Tubers showing typical Sprain—the progeny of a healthy tuber planted in infected soil.

Fig. 5. Tubers showing Corky Bacteriosis of the xylem, but no Sprain—the progeny of a tuber taken from a crop infected with both diseases planted in sterile soil.

Fig. 6. A tuber showing typical Sprain spots—the progeny of a healthy mother tuber grown in soil inoculated with the organism *B. rubefaciens.* 1925 experiment.

Fig. 7. The same as for Fig. 6. 1926 experiment.

Fig. 8. Microphotograph of a Sprain spot produced by inoculation with the organism *B. rubefaciens.* 1926 experiment. (*a*) Parenchyma cells still packed with starch, (*b*) empty cells, (*c*) cork layer. The spot was in the cortex of the tuber and should be compared with the naturally produced spot shown in Text-fig. 1.

Fig. 9. Tubers showing Corky Bacteriosis—the progeny of healthy tubers grown in soil inoculated with mixed cultures of *B. rubefaciens* and *B. suberfaciens.* Only the latter organism appears to have taken in this infection.

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THE BIOLOGY OF OAT SMUTS

I. VIABILITY OF THE CHLAMYDOSPORES

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(With 7 Graphs.)

CONTENTS.

I. Introduction.

It would generally be admitted that investigations relating to the control of a disease, whether by the application of remedial measures, or by the use of resistant varieties, are seriously handicapped unless the research worker can produce at will the disease in epidemic form. This can seldom be accomplished until the conditions which govern infection of the host are reasonably well understood.

During the seasons 1922 to 1924 several attempts were made to obtain heavily infected material by which to test the relative efficiency of different fungicides recommended for the control of smut in oats. Quantities of chlamydospores were collected during the growing season and distributed over clean grain of the same varieties from which the spores were obtained. In the case of *Ustilago avenae* (Pers.) Jens., exceedingly heavy contamination of the grain yielded only a low percentage of smutted panicles in the succeeding crop. A similar result was obtained when resistance trials were attempted with *U. avenae*, whereas the same method produced moderately high infection with *Ustilago levis* (K. and S.) Magn (15).

The present biological studies, which arose out of these negative results, had as their ultimate object the elaboration of a technique, whereby the resistance of a variety might be tested in a single season with a good assurance of reliability in the result. Since the chlamydospores are the connecting link between the seasonal attacks of smut fungi it is clearly important that their viability and storage capabilities be studied.

The germination experiments which are described below extended over a period of three years and included spore collections dating back to the 1921 harvest. It has been established that the two species, *U. avenae* and *U. levis*, are not identical in the storage capabilities of their chlamydospores, the latter species having greater longevity than the former. The difference is not so wide as the early experiments indicated, since the spore collections of *U. avenae* first studied were harvested in the field before the crop reached maturity. As the work progressed, the importance of maturity as a factor affecting the viability and longevity of the resting spores became strikingly apparent, and a special technique was found necessary in the case of *U. avenae* to prevent the premature scattering of the fully exposed chlamydospores. The vitality of spores has been changed by exposing them to extremely dry or very moist conditions of storage, but smaller variations in the humidity, such as might normally occur in the laboratory, produced relatively little effect. The difference in germination between one collection of spores and another of the same species appears to depend chiefly upon the age of the sample and the state of maturity when the spores were harvested.

II. HISTORICAL.

The cardinal temperature points have been established for the germination of spores, and for the growth in culture of a large number of species of fungi, including members of the Ustilagineae, but comparatively little attention has been paid to the viability of spores as expressed in their percentage germination. Consequently there is no well recognised technique for carrying out such tests, and different investigators have usually adopted widely different methods in their studies of germination.

Duggar (5) in a physiological study of the spores of several fungi obtained approximate figures for the percentage germination of *Ustilago perennans*, *U. avenae* and *U. striaeformis*, testing them on water, on cane-sugar solution and in the case of *U. avenae* on three solutions of glycerine of varying concentration. The percentage germination was found to be lower in water than in either of the nutrient solutions.

U. striaeformis differed markedly from the two other species in that it gave a germination of only 2 per cent. as compared with approximately 100 per cent. for *U. avenae* and *U. perennans*. The medium in this case was cane-sugar solution. The data show that the percentage germination of the latter species increased considerably from summer to autumn. Duggar refers to the fact that his experience was unlike that of Kühn (9) and Brefeld (3), who were able by the use of suitable nutrient media to induce immediate germination in freshly harvested spores which failed to grow in water.

The details of germination in the spores of certain cereal smuts were worked out by Stakman (16). Tap water, distilled water, and several nutrient solutions were tested, and of these 5 per cent. cane-sugar solution was found to be most favourable to germination. The data are not presented in terms of percentage germination, but notes on the vitality indicate that decided differences exist between the species studied. Thus the spores of *U. tritici* germinated less readily than those of *U. avenae*, *U. nuda* or *U. hordei*. The latter species was found to start germination in a shorter time than any other species examined. *U. zeae* is recorded as giving different results from season to season. At first (1909–10) repeatedly negative results were obtained with this species, but collections made in 1913 germinated quite readily when freshly harvested. Stakman notes the fact that Kühn and Brefeld failed to induce this species to germinate until several months had elapsed, whereas Hitchcock and Norton found no difficulty in germinating freshly collected spores of *U. zeae* in water.

Jones (7) made critical and statistical experiments with the chlamydospores of *U. avenae*, testing the influence of temperature, moisture and lack of oxygen on the percentage germination. The medium adopted after preliminary trials was beef broth (*p*H 6·1). Spores were submerged in the nutrient solution and the germination was studied by the use of Van Tieghem cells. Duplicate tests were made at each temperature and the experiments were repeated fifteen times. The highest figure for germination, namely 29 per cent., was obtained at 21·7° C., the estimation being made after a period of 24 hours' incubation. In the same series of experiments 7·6° C. and 35° C. were established as the lower and upper limits for germination in *U. avenae*.

A table is given showing the time needed at different temperatures to reach the total germination figure. The results indicate that for the particular sample under test, germination was complete at 18° C. in two days, at 22° C. in one day from the time of sowing the spores. The

following figures quoted from Table II illustrate the deterioration of the sample in storage:

Temperature of test (° C.)	Germination after 2 months	Germination after 5 months
16–17	67	21–32
18–19	66	21
„	40	27
20–22	58	19
„	74	14

These results are particularly interesting in connection with certain experiments with *U. avenae* which are described below. Another point which deserves mention is the fact that the spore material used by Jones in the second year gave a higher percentage germination and germinated at a wider range of temperatures than the collection studied in the previous season. The method of harvesting the spores is not described.

The influence of moisture was investigated by sowing spores on dried agar strips held between pieces of filter paper, and burying them in soils of different moisture content. By this method the highest germination results were obtained in relatively dry soil holding moisture equivalent to 30 per cent. of its water holding capacity.

Withdrawing oxygen from the atmosphere was found to inhibit germination.

Novopokrovsky and Skaskin (11) studied the effect of different temperatures on the germination of the chlamydospores of six species of *Ustilago*. They found that the cardinal points for the four temperate species, *U. avenae*, *U. hordei*, *U. nuda* and *U. tritici*, were minimum under 5° C., optimum 20–25° C. and maximum 25–30° C., while for two species which normally grow in a warmer climate, *U. panici-miliacei* and *U. maydis* (*U. zeae*), the corresponding points were 5–10 degrees higher. The results confirm those obtained by Jones with *U. zeae* and *U. avenae*.

The experiments in Russia were carried out in December 1924 with collections of spores which are described as "freshly gathered." The authors draw attention to the fact that with these species no period of rest was necessary for germination. Reference is made to unpublished data which are said to indicate a decrease in germination in direct proportion to the length of time the spores are kept.

In regard to media, five species germinated as readily in tap or distilled water as in nutrient solutions, but *U. tritici* only gave high germination results (69 per cent.) on a synthetic medium.

It is interesting to note that taking the records as a whole the lowest figures for germination were obtained with *U. avenae*.

Interesting results are described by Noble (10) with *Urocystis tritici*, the spores of which have been found difficult to germinate. Various nutrient solutions were tested with very poor or completely negative results. Germination (20–90 per cent.) was finally obtained by sowing the spores on the surface of water and after four days adding to the liquid sections of fresh plant tissue. Adopting this method it was possible to establish the following temperature points, minimum 5° C., optimum 18–24° C., maximum 32° C.

The problem of longevity has received little attention, but Rump (14), who investigated the time required to destroy the chlamydospores of *U. hordei* by moist or dry heat, refers to the fact that in this species viability was maintained for five years. In harmony with earlier investigators he found that no resting period was necessary.

It is evident from this short survey that discrepancies exist in the records of different investigators and in the data obtained with the same species in different seasons. Taking these into consideration it is yet permissible to conclude that members of the Ustilagineae show an interesting lack of uniformity in respect to the germination of their spores. It is not improbable that such differences as exist between the behaviour of spores in closely allied species will prove to be significant in the biological relationship of parasite and host, and the already published data illustrate the need for more detailed study of germination by methods which will give statistical results.

III. Experimental data.

1. *Technique.*

(*a*) *Collection of material.* The material used in the present studies on germination also served as the basis for other investigations relating to the problem of biological specialisation. This fact has influenced the method of collecting and storing the spores. Infected panicles were placed immediately on collection in one or more glazed paper bags, which were spread out to dry on benches in the laboratory before being stored away for the winter.

In the early stages of the work panicles infected by *U. avenae* were harvested before the crop as a whole reached maturity. This seemed at first to be the obvious plan since spores were required in quantity for infection experiments, and in the case of loose smut the spores are readily scattered by wind soon after the panicle emerges from the sheath. It is not improbable that previous investigators have collected *U. avenae* in

the field by a similar method, since the powdery appearance of infected panicles gives one the impression that the fungus has reached maturity. Results show that this is not the case, and in later experiments (1926 and 1927) infected panicles were enclosed on emergence in pollen-proof bags and left on the plant until the normal time of harvesting the crop.

The same method was applied to *U. levis*, although the more compact and better protected spore masses in this species do not necessitate such measures. The excellent germination of samples of this species dating from 1922–5 is partly to be attributed to the fact that infected panicles were harvested only when the healthy plants held ripe grain.

The chlamydospores were finally obtained relatively free from fragments of the host plant by rubbing the dry infected panicles over fine wire gauze by means of a porcelain pestle. The spores were stored in specimen tubes or screw-top jars varying in size according to the bulk of material obtained.

(*b*) *Media used for the germination tests.* Apart from a few preliminary trials with water and soil-extract, the germination tests were carried out exclusively on either 2 per cent. cane-sugar solution (1925–6) or extract of pales (1926–8). The latter medium was prepared as follows after a method suggested by Diehl(4): Five grammes of oat pales were immersed in 150 c.c. of distilled water and heated in the steam steriliser for 1½ hours. After filtering, the extract was poured into test-tubes and sterilised by steaming for 30 minutes on 3 successive days.

Pales from the same bulk of oats, variety Record, were used throughout the entire experimental period, with very uniform results.

Table I shows that in parallel tests this medium gives slightly higher germination figures than 2 per cent. cane-sugar solution. The tests on

Table I.

Showing the influence of nutrients on the percentage germination of chlamydospores.

Species	Date of test	Percentage germination		
		Glass-distilled water	2 % cane-sugar solution	Pale extract
U. levis C 1/25	26. vi. 26	—	62	85
„ „	3. vii. 26	—	69	83
„ „	27. vii. 28	36	70	74
U. avenae L 17	26. vi. 26	—	5	45
„ „	3. vii. 26	—	10	35
„ L 2*m*	27. vii. 28	15	57	68

water proved that the addition of nutrients influences not only the character and behaviour of the promycelium as described by Stakman (16), but also the percentage germination of the chlamydospores in these species. A similar result was obtained by Duggar with *U. avenae*, but it is contrary to the experience of other workers (11). The data are, however, not strictly comparable since different nutrient media are in question.

(*c*) *Temperature and incubation period.* A temperature of 20–22° C. was selected for the tests, since this falls within the range established as the optimum for germination and growth of *U. avenae* (8).

In the summer months of 1925 and 1926 the temperature of the in-

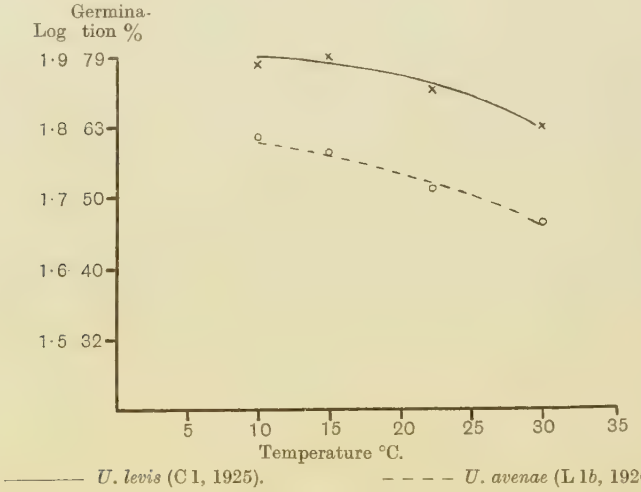


Fig. 1. Graph showing the percentage germination of *U. levis* and *U. avenae* after 2 days at different temperatures. Pale extract, March 1927.

cubator rose on occasions to 25° C., owing to the fact that a system of water circulation was not at that time installed. Such fluctuations suggested the desirability of testing the influence of different temperatures on the percentage germination of spores in *U. levis* and *U. avenae* respectively. The average results of a series of tests on pale extract are represented graphically in Fig. 1. With the particular samples tested the two species showed a closely similar reaction to variations in temperature. In each case the percentage germination fell steadily as the temperature increased from 15° to 30° C. It is evident that the point selected, namely 22° C., does not represent the optimum under the conditions of these tests when the total germination is in question, but

it appeared better to continue the tests at the same temperature rather than to adopt new conditions at that time.

The value of the experiment from the point of view of technique lies in the evidence of parallel behaviour in the two samples, since the experiments under discussion usually involve a comparison between the two species. It is important also to note that a relatively small change occurs from 22° C. to 25° C. Fluctuations in temperature between such limits are not expected therefore seriously to vitiate the results unless a fluctuating temperature as such is a critical factor in the germination of these spores.

Table II.

Showing the percentage germination after incubation at 22° C. *for different periods of time.* 24. ii. 28. *Pale extract* $n = 4$[1].

Species	Reference to spore collection	Percentage germination after 6 hours	12 hours	24 hours	72 hours
U. avenae	L 2*m*/27	46	71	65	67
U. levis	C 1/25	35	73	79	76

With rare exceptions the counts were made after a period of 48 hours' incubation. From data referring to *U. avenae*, already discussed(8), this period appears to be fully adequate for total germination at a temperature of 22° C. This conclusion was confirmed by the writer in certain tests with both species (Table II). The differences between the results at 12, 24 and 72 hours are likely to be errors in sampling (see p. 594).

(*d*) *Method of conducting the tests.* The spores were in all cases germinated on the surface of a solution contained in a covered petri dish measuring 4 cm. in diameter and 1 cm. in depth. A single test was carried out as follows: Five c.c. of a sterilised liquid medium was poured into each petri dish. A sample of the spores to be tested was taken on a platinum loop 1 mm. in diameter and distributed over the surface of the medium as uniformly as possible. Small masses of spores sometimes sank, but the majority floated and formed a fairly uniform layer over the surface of the solution.

To estimate the germination after 2 days' incubation at 22° C., two samples from the edge of the surface film of spores were lifted from each of two duplicate dishes by means of a platinum loop 4 mm. in diameter. Each sample was diluted on a glass slide with two drops of water and examined under a 2/3 in. objective and 6*X* ocular. Observations were made across the centre of the mount by the aid of a moving stage. The

[1] Throughout this paper n is the number of tests averaged.

percentage germination calculated for each slide was based on at least 100 spores. A single test in the present paper means the average of four counts, two taken from each of two duplicate dishes, and is based on a total examination of 500 to 800 spores.

Experience has shown that a very high degree of accuracy is not to be expected from a single test. Fig. 2 shows the results obtained with a sample of *U. levis* (C 1/25) which was tested 44 times over a period dating from June 1926 to March 1928. The sample was used as a control for checking the uniformity of different batches of media and other conditions of the tests, since previous experiments suggested the possibility that a yearling sample of *U. levis* would maintain a high percentage

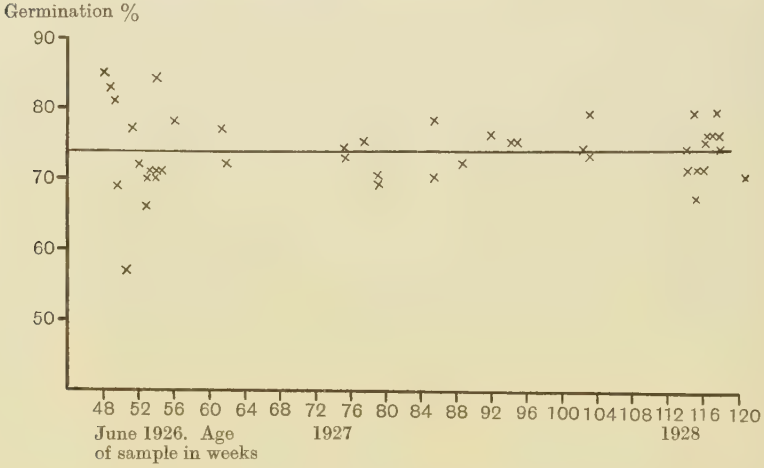


Fig. 2. Graph showing the germination results obtained with a sample of *U. levis* (ref. C 1/25) used as control from June 1926 to March 1928. The sample was 48 weeks old on the first day of test.

germination over a fairly long period of time. The graph shows that the expectation was realised, since the results obviously fluctuated round the mean of 74 per cent. for a period of 72 weeks. It is permissible therefore to examine these data for an indication of the magnitude of the error to be expected in similar tests. Taking the mean as 74 the standard deviation is 4·987. Over 80 per cent. of the readings fall within the limits 68 to 80. The conclusion is drawn from this and from the general body of data that differences of less than 10 are not likely to be significant unless confirmed by repeated tests. In most cases the differences under discussion are at least two or three times this number, and final

conclusions are only ventured upon when the same result has been obtained repeatedly with a wide range of samples.

2. *Single tests on samples from different harvest years.*

The first experiments on the germination of the chlamydospores of *Ustilago* sp. were carried out in the early months of 1925. Collections of spores of *U. hordei*, *U. avenae* and *U. levis*, dating back to the harvest year of 1921 were available at that time. These results (Table III) gave the first indication of a dissimilarity in the longevity of resting spores in *U. avenae* and *U. levis*. Whereas the latter species gave fairly high figures when nearly four years old, the former germinated badly after only 7 months. *U. hordei* was similar in germination to *U. levis*.

Table III includes also the data obtained from the same samples in tests conducted in 1926, 1927 and 1928. Since different media were used the results do not give a true picture of the actual change from

Table III.

Showing the relative longevity of the spores in four species of Ustilago. 1925–1928.

Species	Medium	Date of test	Year in which collections were made: 1921 %	1922 %	1923 %	1924 %	1925 %
U. hordei	Tap water*	ii. 25	—	37	37	55	—
U. levis (C 1)	Rain water	ii. 25	Trace	40	11	25	—
U. avenae	Tap water	ii. 25	0	Trace	3	6†	—
U. levis (C 1)	2 % cane-sugar solution	iii. 25	—	—	—	60	—
U. avenae	,,	iii. 25	—	—	—	6‡	—
U. tritici	,,	21. iii. 26	—	—	—	0	—
U. hordei	,,	21. iii. 26	—	12	26	39	21
U. levis (C 1)	,,	15. iii. 26	2	50	50	47	76
,, (C 1)	,,	20. iii. 26	0	49	45	47	66
U. avenae (L 1)	,,	16. iii. 26	—	—	—	0	4
,, (L 2)	,,	16. iii. 26	—	—	—	0	8
U. hordei	Pale extract	15. iii. 27	—	13	27	39	56
U. levis (C 1)	,,	7. iii. 27	Trace	35	56	36	76
U. avenae (L 1)	,,	9. iii. 27	—	—	—	0	0
,, (L 2)	,,	9. iii. 27	—	—	—	0	Trace
U. hordei	,,	17. iii. 28	—	Trace	10	15	31
U. levis (C 1)	,,	17. iii. 28	Trace	17	42	25	70

* In this case the counts were made after only 26 hours' incubation.

† This figure is the average of results with 8 different spore collections germinating 0–19.

‡ This figure is the average of results with 8 different spore collections germinating 1–14.

Table IV.

Showing the percentage germination of the chlamydospores of U. avenae *and* U. levis *on* 2 *per cent. cane-sugar solution. Spores collected* 1925, *tested for germination* February–March 1926.

Species	Reference to spore collection	Country of origin*	Species and variety of host 1925	Range of figures in 4 counts	Average germination
U. avenae	L 1*a*	Wales	*A. nuda*	2– 8	5
	L 2*a*	U.S.A.	*A. nuda*	8–21	13
	L 2*f*	U.S.A.	*A. sativa* (Victor)	13–23	19
	L 12	Wales	*A. sativa* (Potato)	13–27	20
	L 11	Wales	*A. strigosa*	0– 4	3
Average of 5 collections.					12
U. levis	C 1*a*	Wales	*A. strigosa*	44–61	51
	C 2*c*	U.S.A.	*A. strigosa*	52–61	57
	C 3*b*	England	*A. sativa* (Grey Winter)	48–59	54
	C 4	England	*A. sativa*	44–66	52
Average of 4 collections					53

* All the collections were harvested in Wales in 1924 with the exception of L 2*f* which was obtained by the courtesy of Dr Reed of the Brooklyn Botanic Gardens, direct from the U.S.A. in February 1925.

season to season, but they clearly confirm the difference between the species, at least in so far as these particular samples are concerned. The results prove also that viability can be maintained in *U. levis* and in *U. hordei* for at least as long as $5\frac{1}{2}$ years from the date of harvest. Rump (14) also found germination after 5 years in *U. hordei*, but he did not obtain statistical data.

U. triciti, tested in 1926, gave completely negative results. This species apparently resembles *U. avenae* rather than either of the other species studied. It is, however, probable in view of published data (11) that different results might have been given on other media.

A striking feature of the tests is the low germination of *U. avenae* in samples dating only from the previous season. Table IV shows results obtained in 1926 with a range of collections of the two smuts harvested in 1925. In these the average germination of *U. avenae* was 12 as compared with 53 for *U. levis*, the individual samples in the two species showing relatively close agreement.

The fact should be emphasised that the collections here discussed were made without reference to the stage of maturity in the host plant. The samples of *U. levis* were in all probability more mature than those of *U. avenae*.

3. *Periodic tests on freshly harvested samples.*

Season 1925. During July and August 1925 tests were made at weekly intervals for a period of five weeks and once only after an interval of fourteen weeks (14. x. 25) on three spore collections of the species

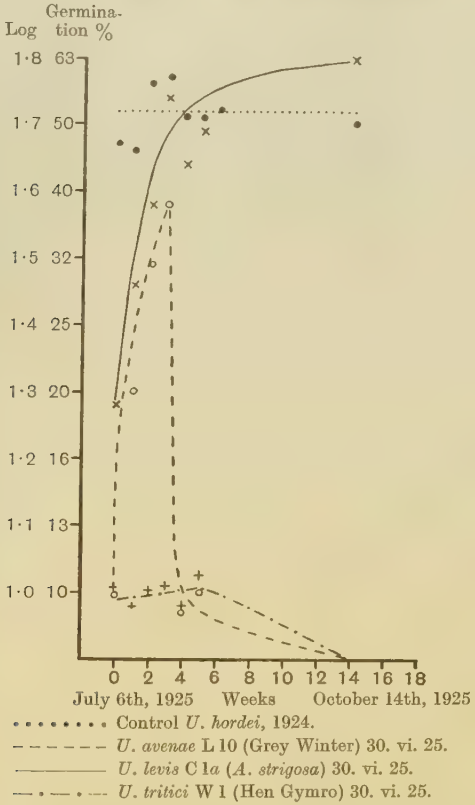


Fig. 3. Graph showing changes in the viability of freshly harvested samples of chlamydospores. 2 % cane-sugar solution, 1925.

U. levis, *U. avenae* and *U. tritici*. To serve as a control of the conditions for germination, a yearling sample of *U. hordei* was tested at the same time. The tests were carried out on 2 per cent. cane-sugar solution at 20 to 22° C. The temperature rose to 25° C. during the incubation period

of the second test, but this did not show any decided influence on the results, which are represented graphically in Fig. 3.

The samples of *U. avenae* and *U. tritici* were collected from farm crops of Grey Winter oats and Hen Gymro wheat respectively. The collection was of necessity made shortly after the smutted inflorescences had emerged from the sheath. The collection of *U. levis* was obtained from pot plants of *A. strigosa* raised in a glasshouse, otherwise, since *A. strigosa* is a late species, it would have been impossible to start all the tests at the same time.

Allowing for the fairly large fluctuations presumably due to errors of sampling, the results obtained with the freshly harvested collections show gradients which are clearly characteristic of the individual samples under test. *U. levis*, starting at 19 per cent. increased in germination to 63 per cent. during fourteen weeks. *U. avenae*, on the other hand, started at 9 per cent., rose to 38 in the fourth week, and fell rapidly to zero by the end of the fourteenth week. *U. tritici* gave consecutively low results, ranging from 6 to 18 during the first five weeks and a completely negative result on October 14th. *U. hordei*, the control sample, gave figures ranging from 46 to 57, with a mean of approximately 52, showing no consistent change during the experimental period.

Season 1926. A similar experiment was carried out in the following year with three samples of *U. avenae*, two of *U. levis* and one of *U. tritici*. The medium used in this case was pale extract, since it appeared to be more suitable than 2 per cent. cane-sugar solution for the oat smuts. It was necessary to conduct the experiment in two parts, series 1 being tested two days later than series 2. Series 1 included the samples *U. avenae* (L 17), *U. levis* (C 1/26) and (C 2/26). Series 2 included *U. avenae* (L 18) and (L 1*a*) and *U. tritici* (W 1). The control sample *U. levis* (C 1/25) was invariably tested with each series. For convenience the two samples of *U. levis* have been graphed with *U. tritici* and the three samples of *U. avenae* are represented in a separate graph (Figs. 4 and 5).

None of the samples of *U. avenae* tested in 1926 gave the ascending part of the curve, indicative of "after-ripening," given by L 10 from Grey Winter in 1927, but each resembled this sample very closely in the rapid fall during weekly tests following in 1927 immediately on the date of harvest. One sample reached zero by October 18th, 1926, one by February 21st, 1927, while the third sample gave a germination of 6 per cent. on the final day of test.

U. tritici, starting with a germination of 20 to 25 per cent., fell to

below 10 per cent. during the first six weeks and gave negative results in February 1927. Both samples of *U. levis* gave curves which are closely similar to that obtained with the same species in 1925. Sample C 2/26,

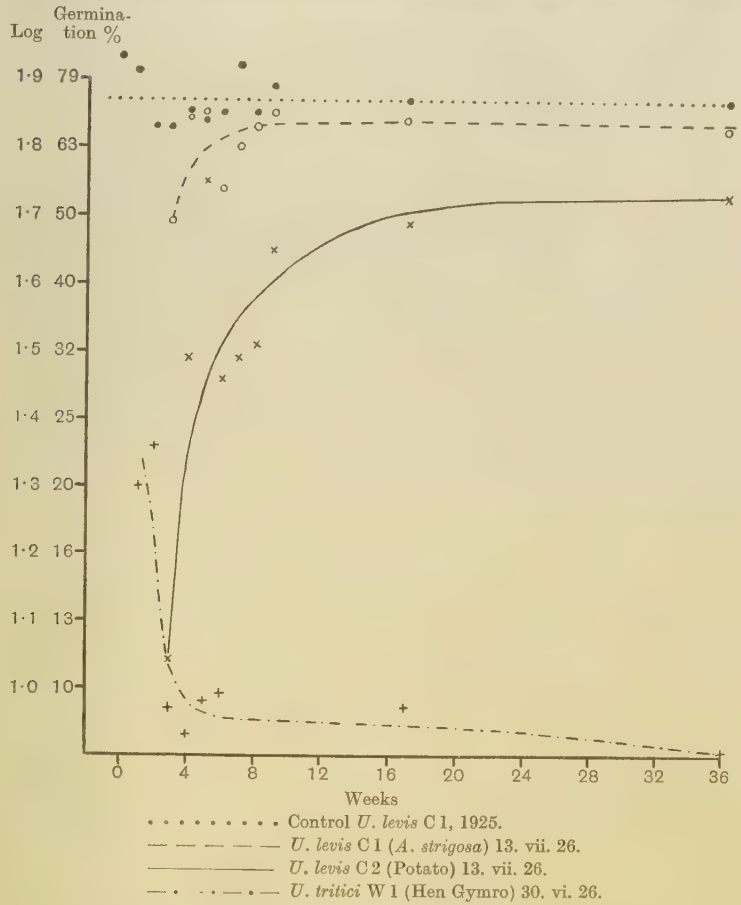


Fig. 4. Graph showing change in the viability of freshly harvested samples of chlamydospores. Pale extract, 1926.

which was known to be harvested from less mature plants, gave consistently lower results than C 1/26, but the curves are clearly of the same type. The behaviour of the control sample, which has been already

discussed (see p. 594), presents a striking contrast to that of the freshly harvested spores.

It is evident that the results of these periodic tests on freshly har-

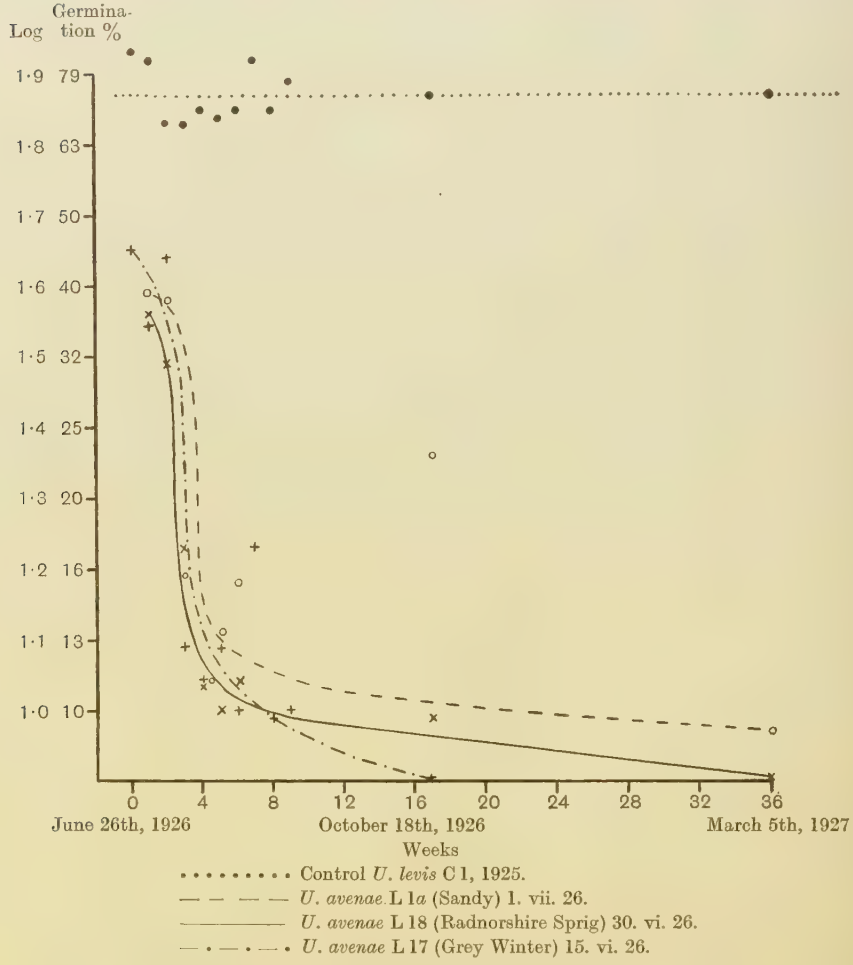


Fig. 5. Graph showing changes in the viability of freshly harvested samples of chlamydospores. Pale extract, 1926.

vested samples are fully in harmony with the data discussed in the previous section, which showed consistent differences between collections of *U. levis* and *U. avenae* when tested seven to eight months from the

date of harvest. Further confirmation was sought in 1927 by testing the germination of nine collections of *U. levis* and *U. avenae* harvested in 1926 but not included in the periodic tests. The results are summarised in Table V.

Four collections of *U. levis* gave germination results ranging from 49 to 62 per cent., 3 collections of *U. avenae* ranged from 2 to 14 per cent. Such results are in close agreement with previous experiments. Two collections of *U. avenae*, L 1*b* and L 2*b*, germinated at 65 and 62 per cent. respectively, results which are higher than any previously obtained with this species even during the summer months.

It appeared at first as if the discrepancy might be caused by the fact that samples which gave the higher germination were stored as panicles in glazed bags until February 1927, whereas L 11*a* and L 12*a* were rubbed through sieves in July of the previous year. This seemed improbable, however, when L 12*b*, which was not sieved until March 1927, gave a result of only 13 per cent.

Further reference to the data relating to these samples showed that the two lots of *U. avenae*, germinating at over 60 per cent., had been covered by pollen-proof paper bags, and were harvested at a later date than any other samples of *U. avenae* previously studied.

Table V.

Showing the germination of nine collections of U. levis *and* U. avenae *harvested on different dates in* 1926. *Tested February to March* 1927 *on pale extract at* 22° C.

Reference to spore collection	Species and variety of host	Date harvested	Conditions of harvesting	Date sieved	Germination 26. ii. 27 to 3. iii. 27 $n=4$
U. levis					%
C 1*e*	*A. strigosa*	—	Harvested with ripe crop	22. ii. 27	62
C 2*d*	*A. sativa* (Victor)	27. vii. 26	Bagged on emergence	22. ii. 27	55
C 3*a*	*A. sativa* (Potato)	27. vii. 26	„ „	18. ii. 27	51
C 4*a*	*A. sativa* (Potato)	27. vii. 26	„ „	11. ii. 27	49
U. avenae					
L 1*b*	*A. sativa* (Sandy)	28. vii. 26	„ „	25. ii. 27	65
L 2*b*	*A. sativa* (Sandy)	28. vii. 26	„ „	26. ii. 27	62
L 11*a*	*A. strigosa glabrescens*	10. vii. 26	Not bagged	14. vii. 26	2
L 12*a*	*A. sativa* (Potato)	10. vii. 26	„	14. vii. 26	14
L 12*b*	*A. sativa* (Potato)	10. vii. 26	„	2. iii. 27	13
Control C 1/25	—	—	—	—	70–74

These considerations led to the inception of certain experiments involving different methods of storage and varied dates of harvesting, which are discussed in the paragraphs below.

4. *The influence of harvesting and storage conditions on the viability of chlamydospores in* U. levis *and* U. avenae.

(*a*) *Varied conditions of atmospheric humidity.* Samples C 1*e* of *U. levis* and L 2*b* of *U. avenae* were selected, since they showed a similar germination (62 per cent.) after a period of eight months in store.

On April 5th, 1927, small open petri dishes containing 0·25 gm. of spores from the bulk samples were placed in small desiccators holding the following solutions[1]:

	%	Corresponding vapour pressure at 15° C.
A. Concentrated sulphuric acid	99·90	Practically nil
B. Solution of „	57·65	2·674
C. „ „	43·75	6·194
D. „ „	33·10	8·995
E. Water	—	12·728

The desiccators were placed in the dark at room temperature. The acid solutions were renewed once during the experimental period of 48 weeks.

The samples in dessicator E, standing over water, were soon covered by a thick felt of mould, and this unit was therefore discarded. Microscopic mounts showed that the chlamydospores had not germinated in the saturated atmosphere.

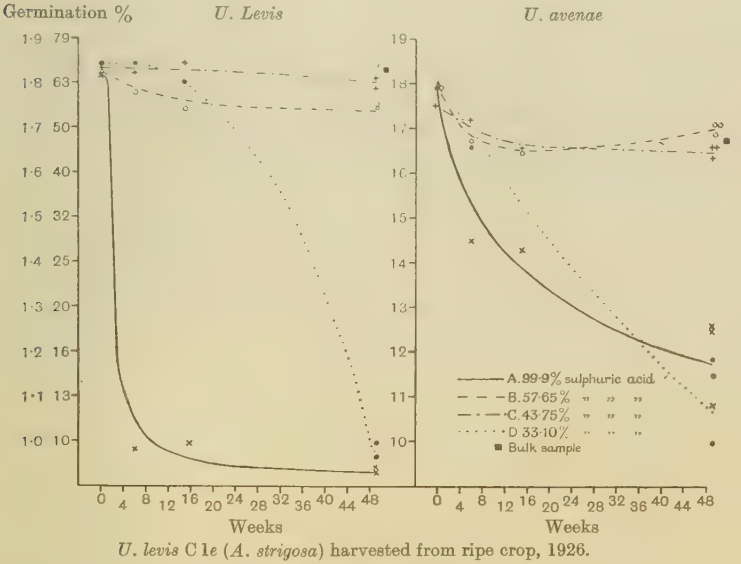
At a later date, May 18th, 1927, sample D of *U. levis* also showed signs of mould, and eventually both lots of spores in desiccator D became coated over with a sparse growth of *Penicillium* sp. Tests were continued until March 1928 in spite of the difficulty of sampling these lots. The results (Fig. 6) show the damaging effect of moist conditions on the viability of spores in store.

At the end of the experiment each bulk sample was tested for comparison with the samples stored under controlled conditions of humidity. The conclusions may be summarised as follows: In both species exceptionally dry as well as exceptionally moist conditions had a depressing effect on germination. This was particularly marked in the covered smut,

[1] The author is considerably indebted to Mr T. W. Fagan, M.Sc., F.I.C., Advisory Chemist, for supplying the solutions of sulphuric acid.

the germination of which fell from over 60 to under 10 per cent. during the first 6 weeks in storage over pure acid.

Units B and C, which provided more normal conditions, produced little change in viability. In the case of *U. levis* the drier atmosphere of B appears to have lowered the germination to a very slight extent, whereas C agreed closely with the bulk sample, showing no appreciable fall during a period of 48 weeks.



U. levis C 1e (*A. strigosa*) harvested from ripe crop, 1926.
U. avenae L 2*b* (Sandy) bagged and harvested July 28th, 1926.

Fig. 6. Graph showing changes in viability resulting from different conditions of atmospheric humidity. Germination tests on pale extract, April 5th, 1927, to March 16th, 1928.

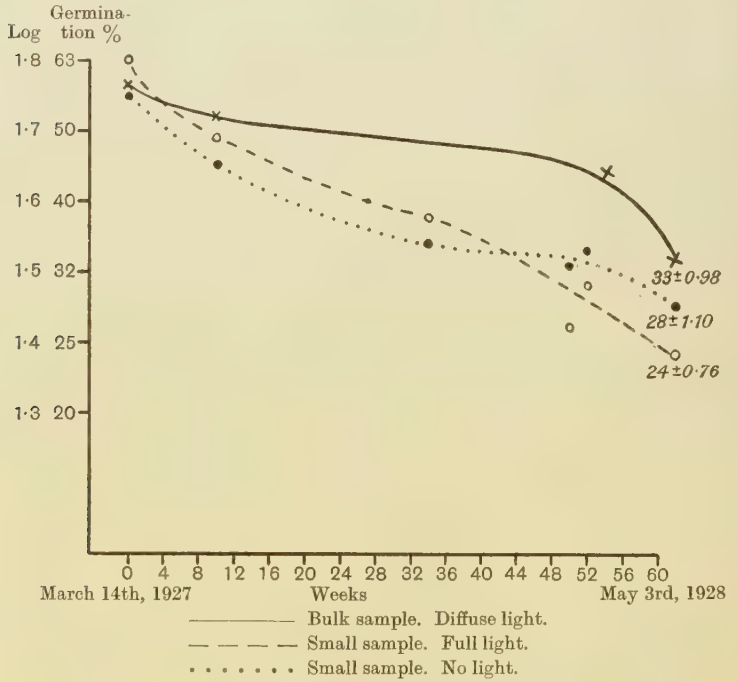
With *U. avenae*, units B and C gave no consistent variation, and in the final test they do not differ appreciably from the bulk sample. Irrespective of slightly different storage conditions the germination of the loose smut fell from 60 to a figure lying between 46 and 50 per cent.

The data as a whole indicate that slight changes in humidity, which might occur from inequalities of corks or stoppers are not likely to influence to a serious extent the viability of spores in storage.

(*b*) *Darkness versus light.* Two samples, each weighing 0·2 gm., were taken from a collection of *U. avenae* (L 1*b*) which gave a germination of

65 per cent. in February 1927 (Table V). The samples were placed in corked specimen tubes, one of which was covered with black photographic paper. The tubes were left near a south-east window for 60 weeks, while the stock sample was stored in diffuse daylight at the back of the room. Results of periodic tests on the three samples are shown in Fig. 7.

All samples decreased in germination during the experimental period, but the loss in viability was most pronounced in the sample



The final figures shown on the graph represent the average of 4 tests (20 counts) conducted during the period April 16th to May 3rd. The sample receiving full light gave the lowest figure every time; the bulk sample gave the highest figure in 3 tests; it was once beaten by the sample receiving no light.

Fig. 7. Graph showing the percentage germination of three samples of *U. avenae* (L 1*b*) under different conditions of storage, March 1927 to May 1928.

receiving direct sunlight and least in the bulk sample. It is probable, since humidity and temperature were not controlled, that the change was mainly due to secondary effects resulting from a drier atmosphere in the tube exposed to full and direct light. The results indicate that only

small changes are to be expected as the result of storing samples in different intensities of subdued daylight[1].

(*c*) *Date of harvesting and method of storing spores.* In July 1927, eight collections of panicles infected by different biological species of *U. levis* and *U. avenae* were divided into two parts which received the following treatment:

(1) Panicles stored in glazed paper bags in diffuse light. Sieved and tested for germination January 1928 (*Z*).

(2) Panicles rubbed and sieved July 29–31, 1927.

Samples of spores (0·2 gm.) were placed on watch glasses *X* and *Y*, and tested for germination August 1–5. Series *X* was exposed to the atmosphere of the laboratory. Series *Y* was stored in a desiccator containing 43·75 per cent. sulphuric acid, a solution corresponding to *C* in the previous experiment. Series *X* and *Y* were again tested for germination with series *Z*, at the end of January 1928. The results are summarised in Table VI.

A comparison of the first two columns shows that samples *X* and *Y* did not differ widely before they were put in their respective places of storage. The maximum difference was 11 and the average figures for eight samples were 54 and 53 per cent. The January tests gave average figures of 54, 55 and 54 for *X*, *Y* and *Z* series respectively, with a maximum difference of 6. It is clear that the different methods of storing the spores exerted no appreciable influence on the viability. It is permissible, therefore, to ignore storage conditions and to average the data for August and January. It is then evident that the behaviour of samples in store is linked with the date of harvesting. The highest results in August and in January, with both species of smut, were given by collections taken from mature plants.

Comparing the two species we find apparent differences. The germination of *U. levis* increased from August to January irrespective of the relative maturity of the host plant. This result confirms those obtained in 1925 and 1926 in experiments which involved periodic tests.

U. avenae gave more variable results. Samples L 11*C* and L 12*E*, harvested from immature crops without "bagging," decreased during the storage period, and the same is true of sample L 11*E*, although this was collected when the host was apparently mature. Sample L 1*D* did not differ appreciably on the two dates of testing, while sample L 2*Q*

[1] The greater mass of the bulk sample would also retard drying and might possibly influence the storage capacities of the spores. The experiment is inconclusive in respect of the action of light rays on the viability of chlamydospores.

showed a slight increase in January. These samples were both harvested late and were presumably mature.

Although one sample of *U. avenae*, namely L 11*C*, dropped in germination to 22 per cent. in January, none gave such low results as were obtained in previous trials with this species. Further tests were carried out on 12 more samples of loose smut, collected in the glasshouse or in the field. The latter give a better comparison with previous results since this was the method of collection originally adopted.

The results given in Table VII show in a decisive manner that the germination capacity of a sample is influenced by the date of harvesting. The six samples of series 1 represent three biological species of *U. avenae*, harvested early (not bagged) and harvested late (bagged). The range of figures for the former is 8 to 17 per cent. and for the latter 50 to 62 per cent. The range of results in 6 lots harvested in the field is 1 to 20 per cent.

(*d*) *Germination of spores of* U. avenae *obtained by gently shaking the exserted panicles.* A collection of spores was made by this method on June 11th and 12th, 1927, from plants in a glasshouse. One half was stored in a corked specimen tube and the other half in an open petri dish over 43·75 per cent. sulphuric acid. Germination tests were made at the beginning of the experiment and again after periods of 4, 18 and 34 weeks. The results are shown in Table VIII.

Both samples fell in germination at a rapid rate, the one giving under 0·1 per cent. germination on October 18th, 1927, and the other almost negative results on February 13th, 1928. Allowing for the fact that tests were made infrequently, it is evident that this sample behaved in a way which recalls certain collections of *U. avenae* represented in Figs. 3 and 5.

It is apparent that storing over acid to give moderate and uniform humidity failed to prevent the rapid loss in viability.

The behaviour of these spores, which might reasonably be expected to be not far from maturity, suggested the possibility of spores in different regions of the spikelet varying in potential longevity. For example, the inner zones of chlamydospores might possess the power of remaining viable for a longer period than those on the surface, which are the first to be scattered. The following results obtained in February 1928 however negative this view:

(1) Spores from the bottom of a glazed bag in which panicles were enclosed while on the plant. Germination 62 per cent.

(2) Spores obtained by shaking the same panicles in February 1928. Germination 67 per cent.

Table VI.

Showing the germination in August and in January of samples of spores of U. avenae *and* U. levis *harvested on different dates. Germination tests on pale extract,* 1927–8.

Reference to spore collection	Tested August 1927: X. Exposed to air of laboratory	Tested August 1927: Y. Over acid in desiccator	Tested January 1928: X. Exposed to air of laboratory	Tested January 1928: Y. Over acid in desiccator	Tested January 1928: Z. Rubbed and sieved Jan. 1928	Average August tests $n=8$	Average January tests $n=12$
A. Panicles harvested before crop reached maturity.							
U. avenae							
L 11*c*	40	47	22	21	24	43 ± 2·6	22 ± 0·6
L 12*e*	42	52	39	42	35	47 ± 3·0	39 ± 1·3
U. levis							
C 2*n*	37	41	58	57	54	39 ± 2·9	56 ± 1·5
B. Panicles bagged and harvested when mature.							
U. avenae							
L 11*e*	63	58	44	46	41	60 ± 2·3	44 ± 3·0
L 1*d*	68	57	63	59	59	62 ± 2·0	60 ± 1·4
L 2*q*	44	48	58	60	66	46 ± 2·4	61 ± 1·3
U. levis							
C 1*h*	69	62	74	80	75	66 ± 1·9	76 ± 1·6
C 2*h*	64	59	74	76	77	61 ± 2·0	76 ± 1·6
Average	53·6	53·0	54·0	55·1	53·9		

Table VII.

Showing the germination of twelve collections of U. avenae *harvested on different dates* 1927. *Sieved February* 1928 *and tested on pale extract at* 22° C.

Reference	Variety	Date harvested	Expt. number	Place of experiment	Method	Germination % $n=4$
U. avenae. Series 1.						
L 1*a*	Victor	2. vii. 27	C 166	Glasshouse	Not bagged	17
L 1*c*	,,	28. vii. 27	,,	,,	Bagged	57
L 2*b*	Potato	10. vi. 27	C 184	,,	Not bagged	14
L 2*m*	,,	19. vii. 27	,,	,,	Bagged	62
L 11*a*	*Orkney strigosa*	2. vii. 27	C 166	,,	Not bagged	8
L 11*d*	,,	28. vii. 27	,,	,,	Bagged	50
Control						
C 1/25	*A. strigosa*	3. viii. 25	C 193ii	Field	Not bagged	71
U. avenae. Series 2.						
L 11*g*	*A. strigosa glabrescens*	12. vii. 27	C 190	Field	Not bagged	20
L 13	Earl Haig	9. vii. 27	C 183	,,	,,	1
L 14	Sandy	9. vii. 27	,,	,,	,,	15
L 15	Radnorshire Sprig	9. vii. 27	,,	,,	,,	12
L 16	Ceirch du bach	12. vii. 27	,,	,,	,,	11
L 17	Marvellous	8. vii. 27	,,	,,	,,	5
Control						
C 1/25	*A. strigosa*	3. viii. 25	C 193ii	,,	,,	76

Table VIII.

Showing the loss of viability in storage of chlamydospores of U. avenae L 2/27 *obtained by gently shaking exserted panicles,* 11. vi. 27. *Germination test on pale extract.*

Date of test	Sample 1. Corked specimen tube	Sample 2. Stored over 43·75 % sulphuric acid	Control *U. levis* C 1 1925
13. vi. 27	59	59	72
16. vii. 27	45	25	76
18. x. 27	Trace	6	74
13. ii. 28	0	Trace	75

(3) Spores obtained from the interior of spikelets on the same panicles. Germination 62 per cent.

It is concluded, therefore, that the spores obtained by shaking the panicles in June 1927 lost their viability because they were immature, and the only safe method, so far devised, of obtaining samples of *U. avenae* with a high germination capacity, is that which involves covering the panicles with bags and collecting the chlamydospores as they naturally reach maturity.

IV. Discussion of results.

The conclusions arrived at in the previous pages may be summarised briefly as follows:

U. levis. A short period of "after ripening" was observed in samples harvested before the host plant was completely mature. The data show that in general the chlamydospores of this species reach a maximum germination figure during the autumn months of the harvest year and maintain a high germination capacity over a long period. In one sample, periodically tested, no falling off was evident in $2\frac{1}{2}$ years. In other samples viability was proved to last for at least $5\frac{1}{2}$ years.

U. avenae. The limits of longevity have not been established for this species, since prior to 1926 the samples under study were harvested in an immature condition. There is, however, distinct evidence that samples harvested with special precautions against premature dispersal suffered a small but definite decrease in viability during the second year in store.

Immature samples collected from infected panicles shortly after their exsertion reached their maximum germination figure almost im-

mediately, dropped rapidly during August and September, and invariably gave very low results when tested in the spring of the following year.

U. hordei and *U. tritici.* These species were included in only a few experiments. The results prove that *U. hordei* in the matter of longevity resembles *U. levis.*

High germination figures were never obtained by the writer with *U. tritici,* perhaps owing to the use of media unsuited to that species. The low results and the rapid loss of vitality in storage may also be related to the immaturity of the spores, since the method of "bagging" infected inflorescences, successfully used with *U. avenae,* was not tested with the loose smut of wheat.

It has been shown that the vitality of spores in *U. levis* and in *U. avenae* was influenced by subjecting them to certain extreme conditions of storage, but that small changes in the method of keeping spores during the winter months produced little or no effect. The data as a whole emphasise the importance of maturity as a critical factor in determining the germination capacity of any particular sample of spores. It is suggested that discrepancies in the results of previous investigators may perhaps be explained by differences in the maturity and in the age of samples tested for germination. It is evident that in the case of *U. avenae* considerable variations may be expected from spores of the same biological species, growing on the same host, in the same field, but collected on different days. During calm weather the spores are likely to remain longer on the panicle and to attain to a higher state of maturity, but even so, to depend upon field collections for viable spore material must involve a certain amount of risk.

These results have a distinct bearing on the interesting researches of Zade (18, 19) and others (1, 4, 13) on the details of infection of the host by *U. avenae.* Zade showed that chlamydospores of this species will produce under favourable conditions, gemmae and mycelia, and that these can retain their viability during the winter months and will infect the shoot when the grain germinates. If this is the natural method of contamination and infection in *U. avenae,* the germination capacity of chlamydospores during the summer months becomes a matter of paramount importance, and their longevity a point of secondary significance under ordinary field conditions. It is evident from the data here presented that spores carried to the stigma or falling between the pales at flowering time are likely to be immature. Such spores are capable of immediate germination but are not likely to remain viable for a long

period. The chances in favour of successful invasion of the host will be greater if the sequence of events is that described by Zade.

With *U. levis* the case is different. The compact and covered masses of chlamydospores remain more or less intact until harvesting and thrashing operations provide the opportunity for contamination of the grain. Infection may be expected to follow the germination of chlamydospores in autumn or spring. Thus the long maintained viability of the resting spores in this species can be correlated with the accepted procedure of contamination and infection.

It is evident from published papers that other workers have experienced difficulty in obtaining good infection with loose smut of oats. Low results are no doubt frequently due to unsuitable temperature or moisture conditions in the soil at the time of germination, since these are known to be factors of great importance (2, 6, 12, 17). It is not impossible that in some cases the viability of the spore material was poor, and that this contributed to produce the unexpectedly low results sometimes obtained. Further reference to this will be made in a later paper dealing with infection experiments conducted with the spore collections which formed the basis for this work.

V. Summary.

1. The viability of the chlamydospores of *U. avenae* and *U. levis* has been under investigation since 1925. Samples of spores were tested dating back to 1921. The results are expressed in terms of percentage germination. Tests were carried out for the most part on 2 per cent. cane-sugar solution or on an extract made from the pales of oats.

2. Samples of *U. levis* showed a short "after ripening" period. They reached a maximum germination figure about two months after harvest and showed no loss in viability during a period of 2 to $2\frac{1}{2}$ years. Some samples were viable after $5\frac{1}{2}$ years.

3. Panicles of *U. avenæ* collected soon after exsertion yielded immature spores which quickly lost their viability. Mature samples, collected by covering the panicles with pollen-proof bags, showed only a slight loss in viability after nearly 2 years in storage.

4. The influence on viability of several methods of storage was investigated.

5. The data emphasise the importance of maturity as a critical factor in the viability of samples. Divergent results obtained with the same species from time to time are probably to be explained by differences in the maturity and age of the samples tested.

6. The significance of the results in connection with the different methods of contamination and infection current in the two species of oat smut is discussed.

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VI. REFERENCES.

(1) ARLAND, A. (1924). Der Haferflugbrand, *Ustilago avenae* (Pers.) Jens. Biologische Untersuchungen mit besonderer Berücksichtigung der Infektions- und Anfälligkeitsfrage. *Botanisches Archiv*, VII, 70–111.

(2) BARTHOLOMEW, L. K. and JONES, E. S. (1923). Relation of certain soil factors to the infection of oats by loose smut. *Journ. Agr. Res.* XXIV, 569–75.

(3) BREFELD, O. (1883). Botanische Untersuchungen über Hefenpilze. *Die Brandpilze*, I, 68. Quoted from (5).

(4) DIEHL, O. (1925). Experimentelle Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes. *Botanisches Archiv*, XI, 146–99.

(5) DUGGAR, B. H. (1901). Physiological studies with reference to the germination of certain fungous spores. *Bot. Gaz.* XXXI, 38–66.

(6) JOHNSTON, C. O. (1927). Effects of soil moisture and temperature and of dehulling on the infection of oats by loose and covered smuts. *Phytopathology*, XVII, 31–6.

(7) JONES, EDITH SEYMOUR (1923). Influence of temperature, moisture and oxygen on spore germination of *Ustilago avenae*. *Journ. Agr. Res.* XXIV, 577–91.

(8) —— (1923). Influence of temperature on the spore germination of *Ustilago zeae*. *Ibid.* XXIV, 593–7.

(9) KÜHN, J. G. (1859). Die Krankheiten der Kulturgewächse, p. 70. Quoted from (5).

(10) NOBLE, R. J. (1923). Studies on *Urocystis tritici* Koern, the organism causing flag smut of wheat. *Phytopathology*, XIII, 128–39.

(11) NOVOPOKROVSKY, I. V. and SKASKIN, F. D. (1925). Effect of temperature on the germination of the chlamydospores of cereal smuts (genus *Ustilago*). Pamphlet of the North Caucasus Regional Land Administration Rostoff-on-Don. 28 pp. (English summary.)

(12) REED, G. M. and FARIS, J. A. (1924). Influence of environmental factors on the infection of sorghums and oats by smuts. *Amer. Journ. Bot.* XI, 502–12, 579–99.

(13) RÖSCH, A. (1926). Studien über den Haferflugbrand, *Ustilago avenae* (Pers.) Jens. und den Glatthaferbrand, *Ustilago perennans* Rostr., mit besonderer Berücksichtigung der Immunitätsfrage beim Haferflugbrand. *Botanisches Archiv*, XIII, pp. 384–431.

(14) Rump, L. (1926). Studien über den Gerstenhartbrand (*Ustilago hordei* Kell. u. Sw.). Forsch. auf dem Gebiet der Pflanzenkrank. und der Immunität im Pflanzenreich, II, 21–76. *Rev. Appl. Myc.* v, 604. 1926.

(15) Sampson, Kathleen (1925). Some infection experiments with loose and covered smuts of oats, which indicate the existence in them of biological species. *Ann. Appl. Biol.* XII, 314–25.

(16) Stakman, E. C. (1913). Spore germinations of cereal smuts. University of Minnesota Agricultural Experiment Station, Bulletin 133. 77 pp.

(17) Tråen, A. E. (1925). Über den Einfluss der Temperatur und der Feuchtigkeit auf den Brandbefall des Hafers durch gedeckten Haferbrand (*Ustilago laevis* (K. u. S.) Mag.). *Meldinger fra Norges Landbrukshøiskole*, v, 157–68.

(18) Zade (1922). Experimentelle Untersuchungen über die Infektion des Hafers durch den Haferflugbrand (*U. avenae* Jens.). *Frühlings Landw. Zeit.* LXXI, 393–406.

(19) —— (1924). Neue Untersuchungen über die Lebensweise des Haferflugbrandes. *Angew. Bot.* VI, 113–25. *Rev. Appl. Myc.* III, 642. 1924.

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AUTOCATALYSIS AND GROWTH

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(With 3 Text-figures.)

A large amount of data, involving the changes which occur during the growth of many varieties of plants, has been collected by many workers over an extensive period of time. It is only within recent years, however, that any extended analysis has been made of the results from a quantitative point of view. A study of the growth rates of plants and the variations may be expected to yield results of theoretical and economic importance. By means of a quantitative analysis of plant growth an attempt may be made to distinguish between the effects of the "external" and "internal" factors which determine the course of development in an organism such as a plant. The crop-yield of a plant must be dependent upon the combined effects of these two factors, and a knowledge of their relationship is of prime importance in evaluating the effects due to variations in environmental conditions.

In general, it has been found that the accretion of material in the initial stages of growth is slow. This stage is succeeded by a period of rapid growth, and later there is slackening of growth. In the case of plants this last stage is coincident with the approach of maturity. In these circumstances the curve of growth with time is sigmoid in character and can be expressed by means of an equation for an autocatalytic reaction in which one of the products of transformation acts as a catalyst. This has led to the assumption that such growth processes are autocatalysed. The idea has been vigorously developed by many workers, particularly by Robertson(8), who has applied this conception especially in the case of the growth of animals. As Robertson points out, the very simplicity of this relationship constitutes an obstacle to its adoption in expressing the changes in such an extremely complex phenomenon as growth. This author overcomes the difficulty by supposing that the sequence of changes in the development of the organism is dependent upon some "master reaction." This reaction may be regarded as being of such a character as to admit of representation by the equation for

a monomolecular autocatalytic transformation. The idea is an attractive one and has been followed by a number of workers. For example, Gregory (4) has shown that the changes in the lengths and areas of the leaves of *Cucumis sativus* may be closely expressed by means of this formula. Prescott (5) has used this equation to express the flowering curves of Egyptian cotton Reed and Holland (7) showed that, in the case of Helianthus, the deviations from this formula were not significant when it was applied to the increase in height occurring during growth. It has been found (1) that the changes which occur in the sugar content of the juice of ripening grapes may be suitably represented by means of this expression. With a slight modification it may be used to represent the changes which occur during ripening in the soluble-solid content of the juice of grapes and in the total solids in the berry.

Based on the idea that the growth of the organism is autocatalytic in character, Robertson (8) has developed the equation of growth in the form

$$\log x/(a - x) = K\,(t - t_1), \qquad \ldots\ldots(1)$$

where a = the maximum yield of material when the growth cycle is completed, x = amount of material at time t, t_1 = time when $x = a/2$ and K = constant.

The velocity of development is given by

$$dx/dt = kx\,(a - x),$$

where $K = ka$.

The expression in the form given by (1) is the one most suitable for application to the observed data. In the case of plants, if the constants have the significance attached to them by Robertson they should be extremely useful in crop studies. a is the final resultant of the growth processes for the cycle of growth under consideration and, in general, is identical with the final yield of material in the plant where, as a rule, only one growth cycle is evident. According to Robertson, a is dependent upon the average concentration of nutritional material and is therefore affected by the conditions constituting the environment of the growing organism or plant. These conclusions are in agreement with the results obtained in practice. It may be expected that conditions, which are favourable to the growth of a plant, will result in a high crop-yield and consequently a high value for a. On the other hand, Robertson infers that k is a constant which is internal in character and independent of the nutritional level of the tissues. It is, therefore, regarded as a specific inherent constant independent of external en-

vironmental factors. Reed(6) goes so far as to assume that K is a constant apart from a. Since $K = ka$ it is clear that both these views cannot at the same time be regarded as correct. In practice, it has been found that a short growing period is associated with a low crop-yield, *i.e.* a high value of K is associated with a low value of a. It is clear that the value of k must tend to vary in the same way as K and, therefore, a low value of a will be associated with a high value of k. The value of k will therefore be subject to variations in accordance with changes in external conditions.

The results reported by Prescott(5) for the flowering curve of Egyptian cotton show that k is subject to variations according to external conditions. In the case of grapes it has been shown in other papers(2) that changes in locality cause distinct and definite changes in the value of k. For example, the figures given in Table I for the rate of change in the sugar content of grape juice have been obtained for varieties of grapes grown at Paarl and Constantia in South Africa. These two localities are about 35 miles apart and are totally different in soil and climatic conditions. The former has an inland situation, while the latter locality is close to the sea and about $2\frac{1}{2}$ miles from Cape Town.

Table I.

Variety	Locality	a	K	k
White Hanepoot	Constantia	21·8	0·0383	0·00180
	Paarl	24·4	0·0258	0·00105
Barbarossa	Constantia	18·1	0·0427	0·00236
	Paarl	19·5	0·0324	0·00166
Flaming Tokai	Constantia	17·0	0·0435	0·00256
	Paarl	18·2	0·0303	0·00166

These grapes are of the vinifera variety and are three representative samples of table grapes. From Table I it is clear that both a and k are affected by external conditions. Robertson(8) regards k as expressive of the velocity of transformation of unit mass of nutritional materials, and his conclusions regarding the specific constancy of k have been based upon the similarity in the values of k for British and South Australian infants. In the first place, it may be supposed that the evolution of the higher types of animals has been accompanied by an increasing perfection of the various mechanisms which are adapted to maintain constancy of the cell-medium. This would result in a tendency towards a constant growth rate. In cases where the concentration of nutritional materials is altered by changes in external conditions it may be expected that the value for the constant of the velocity of

transformation would also vary. Such a condition might easily arise in the case of plants. On the other hand, it is possible that in the case of animals variations in the effects due to external and internal factors tend to compensate one another. At the same time Prescott's results(5) also bear out the view that k is not an inherent constant which is entirely independent of external conditions.

The conception of a specific inherent constant due to internal factors is an attractive one, and an effort has been made to find some means by which it may be possible to evaluate separately the effects due to the external and internal factors in determining the course of development in the growth of an organism. In a paper entitled "Growth Curves in Relation to Temperature" Crozier(3) has pointed out that there are difficulties connected with the adoption of the simple autocatalytic expression as used by Robertson. For example, it is assumed that the curve of growth is symmetrical about a mid-point of inflection, and the temperature characteristic for the velocity constant K must be constant within a given cycle of growth. There are indications that such may not be the case, and therefore some modification of the expression for the autocatalytic curve of growth becomes necessary. It is supposed that the formation of material is due to a first order transformation in which the product serves as a catalyst for the change. The reaction will therefore be governed by a velocity constant k, proper to it in the absence of x, and also by the velocity constant k_2, due to catalysis by x. The change must therefore be conceived as due to two parallel reactions and the rate of change will be given by

$$\frac{dx}{dt} = (k_1 + k_2 x)\ (a - x).$$

The velocity of formation of x will pass through a maximum value when

$$x = (k_2 a - k_1)/2k_2.$$

If, therefore, any change of condition influences k_1 and k_2 unequally the form of the curve connecting x with time will be changed and the point of inflection will be changed to a new position. If k_1 is of inappreciable magnitude the curve then becomes the same as that used by Robertson. If k_2 becomes smaller the inflection point occurs at an earlier stage.

The integral of the above equation is

$$\log\ (k_2 x + k_1)/k_2\ (a - x) = (k_1 + k_2 a)\ (t - t_1), \qquad \ldots\ldots(2)$$

where t_1 = time when x has a value $(k_2 a - k_1)/2k_2$. The equation in this form is applicable to observed data.

It may be expected that k_1 will be directly affected by changes in external conditions and, therefore, be a measure of these variations. Under these circumstances k_1 may be regarded as the "external constant." On the other hand, k_2 must be dependent upon the amount of transformable material which is present in the tissues of the plant during the processes of growth, and it may therefore be expected that the variations in this constant will be very much smaller than in the

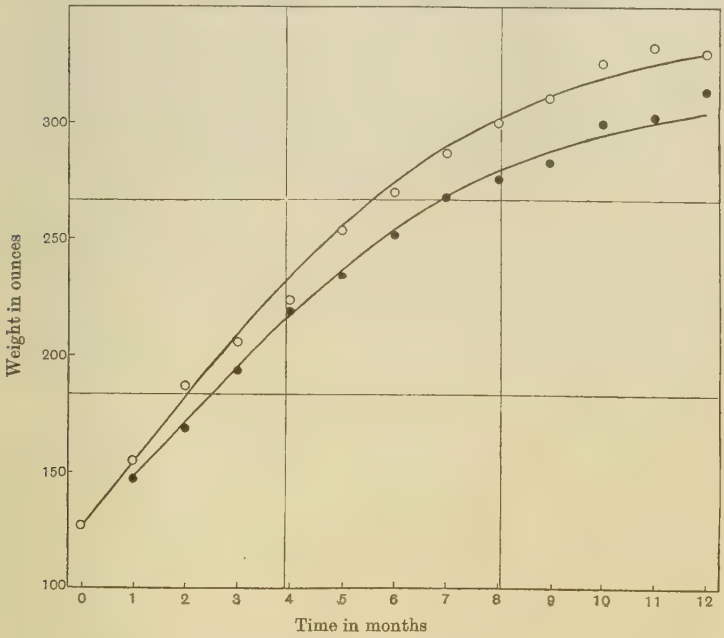


Fig. 1. Curve of growth for infants.

case of the constant k_1. k_2 may therefore be regarded as the "internal constant" and will be a measure of the internal factors governing the growth of the organism. To this extent it may be looked upon as a specific inherent constant. On comparing the equation of growth in the form (1) with that in the form (2) it will be seen that the term ka in the first case corresponds approximately to the term $k_1 + k_2 a$ in the second case. Changes in the environmental conditions will cause a variation in the value of k_1. Since a high value of k_1 will be characteristic

of a short growing period the value of a will be correspondingly small. Under these circumstances it is clear that k_2 may remain substantially constant. If it be assumed as a rough approximation that $ka = k_1 + k_2 a$ the value of k will be practically $k_1/a + k_2$. If a is large and the variations in k_1 are small it is clear from the above assumptions that the value of k will be practically constant. In this way the results obtained by Robertson are explicable, but, at the same time, it is clear that the effects due to environmental changes are masked.

The application of these ideas to the data reported by Robertson[8] in the case of South Australian and British male infants has been investigated. The weights have been given in ounces and the times in months. The results are given in Table II and the agreement between the observed and calculated results is shown in Fig. 1. (Logarithms to the base 10 were used in the calculation.)

Table II.

(*a*) Constants obtained by Robertson.

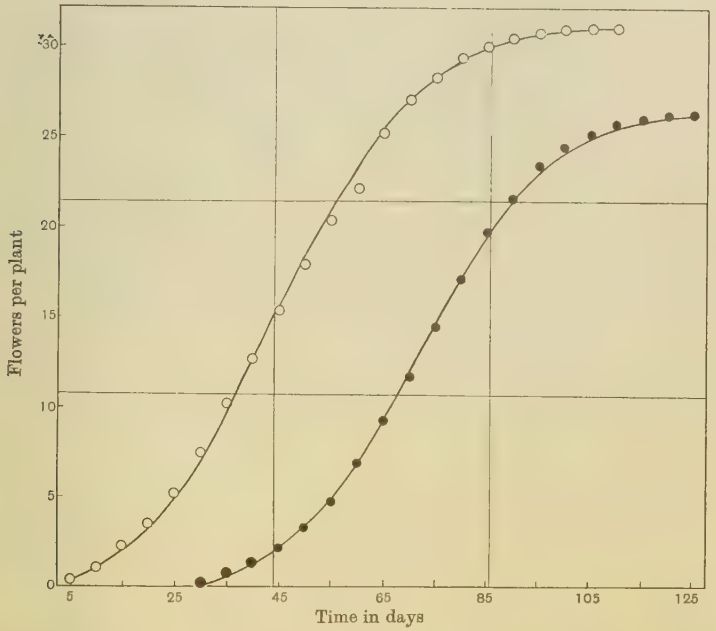
	a	K	k
South Australian	341·5	0·136	0·000399
British	318·0	0·127	0·000398

(*b*) Recalculated constants.

	a	k_1	k_2
South Australian	342·0	0·0105	0·000360
British	318·0	0·0159	0·000340

These results are distinctly interesting since there is clear evidence that the growth processes are affected by environment. It will be seen that the value of k_2 is practically constant, indicating that it has a specific genetic significance. On the other hand, there is an appreciable difference in the values of k_1. It is, therefore, clear that the growth constant is *not* entirely independent of external conditions as supposed by Robertson. The higher value of k_1 in the one case is in agreement with the fact that the value of a is smaller. The results, obtained in the case of plants, do not agree with the supposition that the velocity constant, as defined by Robertson, is entirely independent of environmental conditions. It is only when the effects due to the external and internal factors are considered separately that it becomes possible to attach a definite significance to changes brought about by variations in external conditions. It seems clear from the above results that similar conclusions can be drawn in the case of animals.

In the case of plants the internal mechanism controlling the internal factors of growth cannot be expected to be so highly developed as in animals. For this reason it seems likely that environmental conditions would cause some fluctuation in the values for the "internal" constant, particularly if the conditions were somewhat removed from their optimum value. To obtain some idea of the variation which might be expected when the above conclusions were applied to the growth of

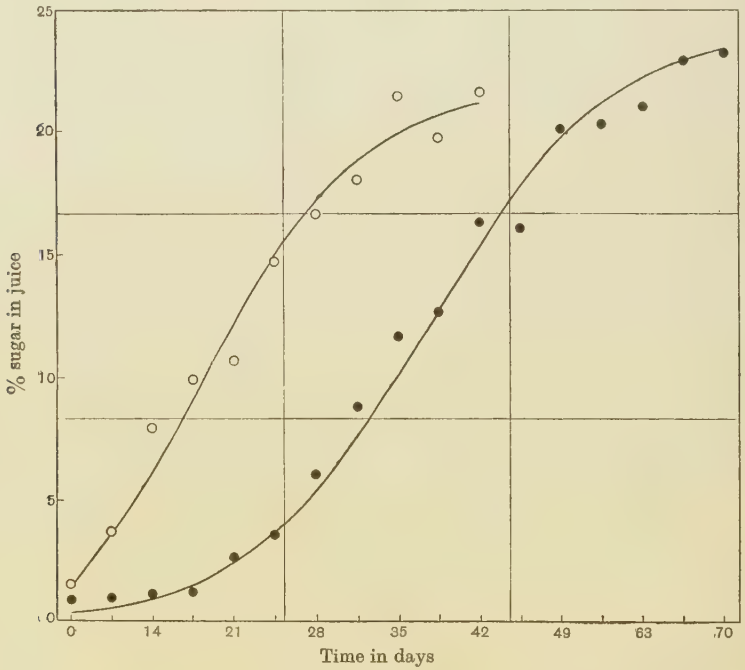


○ = Cotton planted on March 1. ● = Cotton planted on April 1.
The latter curve has been displaced to the right by the addition of 15 days to the times of observation.

Fig. 2. Flowering curve of cotton.

plants, a few examples from recorded data have been worked out. Prescott's figures (5) for the flowering curves of one variety of Egyptian cotton have been employed. The data comprise the results for the variety, Sakellaridis, sown at two different dates, March 1st and April 1st, in the same year. The figures for the sugar content of the juice of two varieties of grapes n.l., White Hanepoot and Barbarossa (2), have been used. In this case the grapes were grown in two entirely different

localities n.l., Constantia and Paarl (South Africa). The results obtained are given in Table III, and the agreement between the observed and calculated results is shown in Fig. 2 for Egyptian cotton and in Fig. 3 for White Hanepoot grapes. (Logarithms to the base 10 were used.)



○ = Grapes grown at Constantia. ● = Grapes grown at Paarl.
Zero time coincides with the commencement of observation in each case.

Fig. 3. Change of sugar content of juice of grapes.

Table III.

Plant	Variations in external conditions	a	k_1	k_2
Sakellaridis	Sown March 1	31·2	0·00120	0·00105
	Sown April 1	26·7	0·00135	0·00120
White Hanepoot	Constantia	22·0	0·00353	0·00125
	Paarl	24·4	0·00014	0·00113
Barbarossa	Constantia	18·1	0·00114	0·00190
	Paarl	19·5	0·00071	0·00178

The corresponding constants recorded by Prescott are: $a = 31{\cdot}2$ and $26{\cdot}6$, $K\,(= ka) = 0{\cdot}044$ and $0{\cdot}049$, $k = 0{\cdot}00109$, $0{\cdot}00143$, while the corresponding values for the grapes are given in Table I.

It is clear from a comparison of the various constants that the value of k_2 is more nearly a specific inherent constant than the value of k (calculated according to Robertson's equation). At the same time it will be seen that the value of k_1 is directly affected by external conditions. For example, k_1 has a higher value for grapes grown at Constantia, and accordingly the value of the crop-yield a is lower in this locality than at Paarl. Similarly, with the Egyptian cotton the effect of later sowing is shown by an increase in the value of k_1 and consequently by a reduction in the crop-yield.

On the whole there seems to be some basis for the assumption that, under normal conditions of growth, the constant k_2 represents the effects of factors which are specific in nature. Where external conditions become extreme there is still the possibility that the internal factors of growth in a plant will be affected. That this may be the case is shown by the slight variations in the value of k_2 in Table III. The results, however, indicate that the variations in k_2 in the case of plants lie within sufficiently narrow limits to justify a closer study of the effects due to external conditions from the point of view of the growth constants. There is no doubt, however, that the value of k in the simple autocatalytic equation does not have the specific significance attached to it by Robertson. The nutritional level of the tissue in the case of animals must be less susceptible to variations in external conditions than in the case of plants. It may, therefore, be expected that the constant k_2 would possess a genetic racial significance of greater value than would be the case with the simple constant k. At the same time the value of k_1 is a more direct measure of the external factors and from this point of view may well repay further study.

Summary.

The autocatalytic equation in the form $\log x/(a-x) = K(t-t_1)$ may be used to express closely the changes which occur during growth.

The constant k, as given by $K = ka$, is not a constant which is independent of environmental conditions.

The changes in growth may be more suitably expressed by means of the equation $dx/dt = (k_1 + k_2 x)(a-x)$. In this case k_1 may be regarded as a constant, dependent upon external conditions, while k_2 is a measure of the internal factors governing the growth processes.

In conclusion the author would like to express his thanks to Dr B. de C. Marchand for the interest he has taken in the preparation of this paper.

REFERENCES.

(1) Copeman (1927). *Trans. Roy. Soc. S.A.* xiv, 267.
(2) —— (1927). *Ibid.* xiv, 395.
(3) Crozier (1926). *Journ. Gen. Phys.* x, 53.
(4) Gregory (1921). *Ann. Bot.* xxxv, 93.
(5) Prescott (1922). *Ibid.* xxxvi, 121.
(6) Reed (1920). *Journ. Gen. Phys.* ii. 545.
(7) Reed and Holland (1919). *Proc. Nat. Acad. Sc. U.S.A.* v, 135.
(8) Robertson. *The Chemical Basis of Growth and Senescence.* London.

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THE ACTION OF SULPHUR AS A FUNGICIDE AND AS AN ACARICIDE. PART I

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(With 2 Text-figures.)

In 1821, John Robertson(28) recommended the use of sulphur as a cure of peach mildew and he concluded that the sulphur "emits powerful effluvia for some time which, it is probable, operate, besides the contact of the substance, in destroying, by its corrosive property, the tender fructification of the mildew." Although over one hundred years have elapsed since these two theories were advanced, they remain to-day unchanged. Cunningham(11), for example, attributed the principal action of sulphur to the emission of certain vapours the nature of which is not known, whilst Eyles(14) stated that sulphur emits fumes destructive to the fungus at temperatures ranging from 75°–100° F. On the other hand, evidence has been adduced by Salmon(29) that in the case of the hop powdery mildew (*Sphaerotheca Humuli* (DC.) Burr.), actual contact of the sulphur particle with the fungus is necessary for fungicidal action.

The fact that sulphur is able, under certain conditions, to exert its fungicidal action without being in actual contact with the fungus or plant has been utilised by Bergmann(6), who introduced the method of painting sulphur on to the hot-water pipes of the greenhouse—a practice which is still widely used in horticulture. That this means of application of sulphur is effective has been established, at least in the case of the mildew *Erisyphe graminis* DC., by Barker, Gimingham and Wiltshire(3).

It became evident that a similar process was at work in the experiments recorded by Lees(18) in which the effect of sulphur upon the black currant gall mite (*Eriophyes ribis* (Westw.) Nal.) was tested. Lees took twigs bearing "Big Bud" and placed them under bell-jars coated on the inside with sulphur applied, in one case, by means of the fumigation process devised by Barker and Wallace(4) and in the other, by spraying the inside of the bell-jar with lime sulphur. In the second case the decomposition of the calcium polysulphides resulted in the

formation of free sulphur from which the soluble calcium salts were removed by washing with water. The mites emerging from the buds on the twigs placed in the sulphur-coated bell-jars were killed, whereas those on the control were unaffected. Lees made no comment on the nature of this action, but it is apparent that the sulphur has here exercised an "action at a distance." As this action may be comparable to that produced in the greenhouse where sulphur has been painted on the hot-water pipes, the black currant gall mite was included in the biological trials to be described later, which followed the chemical part of our investigation.

The main object of our preliminary work has therefore been the investigation of the volatile agent formed when sulphur is applied, not to the plant, but to a heated surface. The factors due to the presence of plant, fungus or acarid are then absent and the problem is simplified. The procedure has been to examine the various proposals put forward, employing chemical means to detect and, if possible, measure the amount of volatile agent formed and to examine the mechanism of its formation. Our intention is to follow up and to attempt the confirmation of the conclusions drawn from chemical tests by using in their place certain fungi and the black currant gall mite.

A large amount of work has already been carried out on this particular problem and it would be well to give, first of all, a summary of the various hypotheses put forward to account for the action of sulphur at a distance. Not only various forms of elementary sulphur but also certain sulphur derivatives, such as sulphur dioxide and other oxidation products and hydrogen sulphide, have been suggested as the agents concerned. Dealing with each compound in turn, we have first:

Sulphur dioxide. Mangini (20) observed that, by the action of ozonised air on sulphur, sulphur dioxide is formed. He therefore considered that under the influence of direct sunlight the plant produces activated oxygen or hydrogen peroxide which is able to oxidise the sulphur to sulphurous acid, the latter then causing the death of the fungus. This view was supported by the work of later investigators, for example, Basarow (5), Mach and Portele (19), who found that on conducting the air surrounding sulphured vines through a solution of sodium hydroxide, sulphuric acid was formed after oxidation with chlorine. Of the factors which govern the rate of formation of sulphur dioxide the latter two investigators found temperature to be of importance, for, whereas at $-3°$ C. the amount of sulphuric acid formed was too small to be determined, at 59° C. the amount formed corresponded to a percentage by weight of

0·0092 of sulphur dioxide. Moritz(25) had previously stated that sunlight acted as an accelerator of the process. This effect of temperature was therefore in accordance with the observations of Marès(22), who found that, whereas at temperatures of 32°–35° C. the destruction of *Oidium Tuckeri* in contact with sulphur was complete in four to five days, the fungus was killed within two days when the temperature reached a maximum of 42° C.

Although the original theory of Mangini required the presence of the plant for the intermediate formation of an oxidising agent, evidence was adduced of the spontaneous combustion of sulphur in air. Moissan(21), by cooling in liquid air sealed tubes containing sulphur and oxygen, was able to detect the presence of sulphur dioxide. The action he found to be much slower in air, but traces of sulphur dioxide were detected at the end of three months when the temperature was maintained at 16°–26° C. There thus arose the tendency to regard the formation of sulphur dioxide from sulphur as a process independent of the plant and fungus and requiring merely suitable conditions of temperature, sunlight and humidity. The statement is found, even in text-books, that gaseous sulphur dioxide evolved is the efficacious agent(23). Bruck(8) attributed the fungicidal properties of sulphur to its power of forming sulphurous acid when exposed to warmth and air. As a corollary of this view, the addition to dusting sulphur of an oxidising agent, such as potassium permanganate or nitric acid, has been recommended by Atherton Lee and Martin(1). The investigators claimed that the efficiency of sulphur employed for the control of eye-spot of sugar-cane (*Helminthosporium sacchari* Butler) was increased by admixture with 0·25 per cent. nitric acid or with 1 per cent. (or better 5 per cent.) potassium permanganate, and stated that these latter substances themselves possessed no fungicidal powers.

On the other hand, many observers have decided against sulphur dioxide as the volatile agent formed from sulphur, mainly for the reasons that sulphur dioxide even at extreme dilutions is injurious to plant tissue and that the gas, if present, would be recognised by its odour. Muth(26), who exposed sulphur in a bell-jar to sunlight throughout the summer, was unable to detect the presence of sulphur dioxide employing starch-potassium iodide as the indicator.

Sulphur trioxide (*sulphuric acid*). Marcille(21) exposed sulphur to sunlight and moisture and found an accumulation, not of sulphur dioxide, but of sulphur trioxide. An examination of various samples of sublimed sulphur revealed the presence of 0·22–0·625 per cent. sulphur

trioxide, to which Marcille attributed their anticryptogamic properties. Blodgett(7) supported the view that the action of sulphur upon the hop powdery mildew is due to its gradual oxidation to sulphur dioxide which, in the presence of water, is further oxidised to sulphuric acid; dilute solutions of both sulphurous and sulphuric acids, he stated, have been shown to destroy the mildew. As recently as 1923, Cockerham(10) in his text-book definitely stated that "Flowers of sulphur...destroys fungi in vineyards by virtue of the traces of sulphuric acid it yields on oxidation."

This theory was supported by the fact that the older workers had found sublimed sulphur more potent as a fungicide than ground sulphur, and it was known that from the latter forms sulphuric acid is generally absent or is present in smaller amount than in the former. The introduction of better methods of grinding has placed ground sulphur and flowers of sulphur in practically the same category as regards fineness of particle, and Goodwin and Salmon(16) were unable to find any important difference in fungicidal efficiency between ground and sublimed sulphur. Moreover, when viewed from the aspect of volatility, although sulphur trioxide boiling at 46° C. would in the absence of water be readily volatilised from the hot-water pipes, it is certain that under normal conditions sulphuric acid would immediately be formed, a substance the volatility of which at temperatures below 100° C. has not been established. It is true that at the temperatures employed in the preparation of sublimed sulphur, the sulphuric acid is probably carried over, a process which may account for its presence in such forms of sulphur.

Pentathionic acid. Young(35), from experiments upon the action of colloidal sulphur prepared in various ways upon fungus spores, attributed the inhibition of germination to the presence of pentathionic acid. He recorded an experiment in which air drawn over flowers of sulphur was shown to contain a volatile compound which, in aqueous solution, reacted with hydrogen sulphide to form free sulphur. This compound was absent in similar experiments in which the air was deprived of oxygen, a result which he claimed afforded definite proof that pentathionic acid is an oxidation product of flowers of sulphur at ordinary temperatures. In addition he stated that pentathionic acid is volatile, a conclusion totally at variance with previous experience. Debus(12) showed the acids of Wackenroder's solution to be non-volatile, whilst attempts to isolate the pure pentathionic acid from its aqueous solution are frustrated by its decomposition above certain concentrations. As

Young advanced no proof of its volatility, it is difficult to see how pentathionic acid can be concerned in the action of sulphur at a distance.

Hydrogen sulphide. The production of hydrogen sulphide by certain yeasts growing in a medium containing free sulphur led Pollacci(27) to suggest that this gas was the toxic agent formed when sulphur is placed in contact with leaf or fungus tissue. It has not, to our knowledge, been suggested that hydrogen sulphide is formed in the absence of vegetable matter, *e.g.* from the sulphur placed upon the hot-water pipes, but the possibility that this gas is the ultimate toxic agent must not be overlooked. Barker(2) was able to show the production of a volatile substance giving the reactions of sulphuretted hydrogen when sulphur is sprinkled on living leaves attached to the parent plant. The formation of hydrogen sulphide from sulphur alone under the action of air, sunlight and humidity was not observed by Vogt(32).

Gaseous sulphur. The volatility of sulphur at ordinary temperature was observed by Sestini and Mori(31), who, although attributing the fungicidal action of sulphur to the formation of sulphur dioxide, sulphurous and sulphuric acids, considered the fungus to be injured not only by contact with the sulphur particles but by the sulphur vapour which they found to be formed in amounts sufficient for detection at 25°–35° C. The blackening of copper placed in a tube containing sulphur was advanced by Hallock(17) as proof of the volatility of sulphur at ordinary temperatures. Although, according to Vogt(32), the rate of volatilisation of sulphur, even in the finest state of division, is extremely small at the highest naturally occurring air temperatures, it is sufficient to produce the characteristic smell of sulphur in sulphured vineyards on which comment has often been made. Salmon(30) recorded that this odour has even been observed in the cooler English hop gardens.

That the fungicidal action of sulphur occurs through the formation of sulphur vapour has been perhaps the view most generally accepted, and this theory has received support from the work of Barker, Gimingham and Wiltshire(3). These investigators showed that the chemically active volatile substance of a reducing character given off from the sulphured hot-water pipes was sulphur itself.

Particulate sulphur. Barker(2) concluded from a continuation of the work mentioned in the above paragraph that the toxic agent formed is not gaseous but solid, and he put forward the hypothesis that sulphur acts in the solid form as finely divided "particulate" sulphur. A direct toxic action of the particulate sulphur was not of necessity stipulated,

but Gimingham(15) suggested that the sulphur in such a form might possess special properties not so evident in the more coarsely divided forms in which sulphur is generally applied.

The agents considered responsible, according to these various hypotheses, for the action of sulphur at a distance may then be limited to:

(i) Gaseous: Sulphur dioxide, Hydrogen sulphide, Sulphur vapour;

(ii) Solid: Particulate sulphur;

whilst the factors thought to be involved in the production of these substances are: Temperature, Oxygen, Humidity and Sunlight.

Experimental: Chemical.

A preliminary series of experiments was carried out in an attempt to measure the rate of formation of the volatile substance by the estimation of the loss of weight of the sulphur. It was evident however that, under ordinary conditions and even at 100° C., the amount of sulphur lost within a period convenient for experiment was too small for accurate determination. More success was encountered by means of the reaction of the volatile material with copper and a method was evolved whereby the stain produced could be quantitatively estimated.

The method of determining the amount of stain produced on the copper was as follows: Electrolytic copper foil was cut into strips of equal size—3 × 2 cm.—washed well with ether to remove grease and stored in alcohol. Each strip immediately before exposure was dipped into a 1 per cent. solution of potassium cyanide for 5 seconds, washed well with tap water and dried between filter paper. After being used, the amount of stain on the copper strip was determined by placing the latter for exactly 5 seconds in a small specimen jar—4 × 2·5 cm.—containing 1 per cent. potassium cyanide. The stain was rapidly dissolved off and, after washing the copper strip with water and drying between filter paper, the strip was used for a repeat determination. A few drops of nitric acid (free from iron) were added to the solution of potassium cyanide, it was evaporated to dryness, the residue taken up with water, a few drops of ammonia added, then boiled down, to drive off the excess of ammonia, to a volume of about 10 c.c. The copper present in the solution was then determined by the colorimetric potassium ferrocyanide method by adding 5 c.c. of 10 per cent. ammonium nitrate and 10 drops of 4 per cent. potassium ferrocyanide, the whole was transferred to a Nessler tube and diluted to 50 c.c. The colour produced was compared with a blank to which a standard solution of

copper sulphate (0·393 gm. $CuSO_4.5H_2O$ per litre) was added from a burette.

The quantity of copper sulphate solution required to bring the colour of the blank up to that of the experiment gives a figure which, after subtraction of the figure for a control test—since copper itself is slowly dissolved by the solution of potassium ferrocyanide—represents the amount of volatile sulphur derivative which produced the stain on the copper.

Two or more trials were carried out at a time in order that comparable results should be obtained while the result itself was taken as the mean of at least four determinations. Other precautions which are noted below under the particular experiment were also taken.

The conditions under which the formation of the copper stain was studied were as nearly as possible those which are regarded as influencing the fungicidal activity of sulphur. The most important of these factors appears to be temperature.

(a) *The influence of temperature.*

In addition to the authorities already quoted (19, 22), Butler (9) considered that temperature is a decisive factor in the fungicidal action of sulphur, whilst Doran (13) stated that the toxicity of sulphur increases with rise of temperature.

For the purpose of estimating the influence of temperature upon the production of the stain-producing sulphur derivative, an apparatus (Fig. 1) was constructed in which two tubes placed side by side were maintained at a constant temperature by immersion in the vapour of a boiling liquid. In each tube was placed a plug of glass wool, one plug (*a*) was dusted with finely ground sulphur and the other left untreated (*b*).

Table I.

Liquid employed	Temp. of inner tube °C.	No. of trials	Duration of trial	Copper dissolved in mg. In sulphur tube	Copper dissolved in mg. In control tube
(i) Water	93	4	5 minutes	0·90	0·29
(ii) Chloroform	58	4	150 „	0·83	0·27
(iii) Carbon tetrachloride	72	4	60 „	0·94	0·31
(iv) Petroleum ether	38–40	—	10 hours*	—	—
(v) Benzene	78	4	40 minutes	0·57	0·26
(vi) Water	93	4	5 „	0·70	0·22

* Only after 10 hours was a stain comparable with (i) obtained.

Air, from outside the laboratory, was drawn at equal rates through both tubes, and the copper strips (*c*) were held by means of clips across the exits of the tubes. The same copper strips were used throughout the series of trials made at any one temperature, but they were interchanged—*i.e.* the strip in the tube containing sulphur was, after washing, replaced in the control tube.

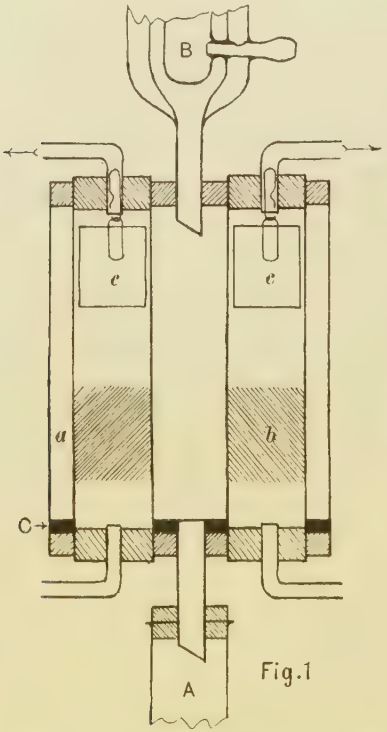

Fig.1

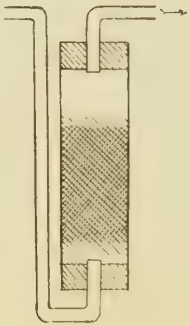

Fig.2

A = Boiling flask. B = Condenser. C = Layer of mercury to protect rubber.

By using different liquids for the vapour bath it was possible to secure a series of constant temperatures which yielded the results shown in Table I.

The figures in the above table clearly show the great influence which temperature exercises on the formation of the stain. If the time required for the production of equal stains on the copper be taken as an index, the amounts of the volatile agent formed at various temperatures are in the following proportions:

Temp. °C.	Amount of volatile agent formed
93	288·0
72	24·0
58	9·6
38–40	< 1·0

It was necessary to show that the interaction of the volatile agent and the copper foil is not influenced by temperature, and for this purpose a similar apparatus was used in which the strips of copper were wrapped round the outside of small glass specimen tubes held by the cork of the exit air tubes. One piece of foil was cooled by circulating water through the small tube.

The result of a quadruplicate trial in which alternate tubes were cooled gave:

Copper foil not cooled	0·44 mg. copper dissolved
Copper foil cooled	0·44 „ „

which shows clearly that the effect of temperature on the production of the stain lies solely in its influence on the formation of the volatile sulphur derivative.

(b) *The influence of oxygen.*

The view that the fungicidal activity of sulphur is due to the formation of oxidation products was supported by Doran(13), who found sulphur capable of inhibiting the germination of fungus spores only in the presence of oxygen. Further, as has been mentioned, Young(35) found the volatile derivative, which he stated to be pentathionic acid, was not formed in the absence of oxygen.

As the accuracy of the method for estimating the relative amounts of the volatile sulphur derivative increases with the rapidity with which the stain is formed it was found advisable, in the experiments described below, to use higher temperatures than would be experienced in actual practice. It will be seen from Table I that the amount of stain formed by the action of sulphur below 60° C. is too small to be measured accurately by the estimation of the copper dissolved by potassium cyanide.

To test the influence of oxygen, two tubes were arranged as in the experiments under (*a*), and equal weights of sulphur dusted on equal weights of glass wool were placed in each tube. Air was drawn through the first tube and carbon dioxide generated from a Kipp's apparatus was passed through the second tube. The air and carbon dioxide were passed through similar wash bottles containing water so as to prevent differences in humidity and to permit the regulation of the rate of flow.

In four trials using steam in the jacket, the strips of copper being interchanged, whilst for two of the experiments the Kipp was connected to the first tube, the amounts of copper dissolved were as follows:

Stain produced in air 0·50 mg. copper
Stain produced in CO_2 0·49 ,,

The presence of oxygen does not therefore appear to influence the production of the volatile sulphur derivative which causes the staining of the copper.

(c) *The influence of humidity.*

Yossifovitch(34) was of the opinion that the action of sulphur against *Oidium* of the vine was diminished by humidity, yet Young(35), in support of his view that pentathionic acid is produced, considered the presence of moisture essential.

Using a similar apparatus to that described in (*a*), the humidity of the air in the two tubes was varied by passing the air in one case through water and in the other through concentrated sulphuric acid. The jacket was heated by steam and the duration of each trial was 5 minutes. Four such trials were made, the wash bottles being changed over for two runs, and the amounts of copper dissolved were:

Stain produced in dried air 0·64 mg. copper
Stain produced in moist air 0·62 ,,

A further set of experiments was carried out in which the jacket was heated by carbon tetrachloride. Each trial was continued for 90 minutes, and from four such trials the amounts of copper dissolved were:

Stain produced in dried air 0·78 mg. copper
Stain produced in moist air 0·71 ,,

The influence of humidity on the production of the stain appears therefore to be negligible although there is evidence that in the absence of moisture its formation is favoured.

(d) *The influence of sunlight.*

The determination of the influence of direct sunlight upon the formation of the volatile sulphur agent producing the stain on copper presented difficulties owing to the absorption of part of the ultra-violet rays by the glass apparatus. It must be remembered, however, that in practice the sulphur painted upon the hot-water pipes of the greenhouse will not be influenced by direct sunlight, and it has not yet been shown that the absorption by the glass of part of the light adversely affects the efficiency of sulphur as a fungicide.

Trials in which one tube of the apparatus used in the previous experiments was darkened by means of a sheet of black paper gave no appreciable difference in the stain produced on the copper foil.

It may therefore be concluded that of the factors suggested as playing a part in the fungicidal activity of sulphur only one, temperature, is of importance in the production of the volatile sulphur derivative producing the stain on the copper foil.

The nature of the volatile substance producing the stain on copper.

In experiments to determine the nature of the volatile substance causing the discoloration of the copper it was found that sulphur dioxide caused no stain under the conditions of experiment. It was therefore necessary to investigate separately the question of the formation of sulphur dioxide, though it may here be recorded that tests with starch-potassium iodide paper failed to detect the presence of sulphur dioxide in the air passed through glass wool dusted with sulphur and heated to 100° C.

Hydrogen sulphide would of course produce an immediate blackening of the copper foil, but the application of the delicate lead acetate test failed to show the presence of sulphuretted hydrogen. It may then be assumed that in the absence of hydrogen sulphide the tarnishing of the copper is due to elementary sulphur either in the form of vapour or in the particulate state. It is comparatively simple to distinguish between these two forms, for whereas the latter is removed by passage of the air through a suitable filter, the former will pass unaffected.

Experiments were therefore carried out for this purpose. The apparatus used consisted of a long tube through which air was drawn upwards; at the bottom end of the tube a wad of glass wool dusted with sulphur was placed and in the middle of the tube a wad of glass wool free from sulphur; at the upper end of the tube a strip of copper foil was suspended. The tube was thus divided into three regions, each of which had an outer jacket through which steam or water could be passed. When carrying out the experiment, steam was passed continuously through the upper and lower jackets and either steam or cold water through the middle jacket.

When steam was used in all three jackets the copper foil tarnished rapidly, whereas if cold water was passed through the middle jacket the stain appeared extremely slowly. This experiment was repeated many times, allowing the air to pass for 15 minutes after the temperature of

the middle jacket had been changed before testing for the presence of volatile sulphur with copper.

The fact that the sulphur was able to pass the heated glass-wool plugs clearly shows that the sulphur is in the form of vapour, whilst the removal of the volatile substance causing the tarnishing of the copper by the cooled glass-wool plug is proof of the absence of hydrogen sulphide. Hence it may be concluded that the sulphur derivative to which is due the discoloration of the copper is gaseous sulphur. No figures have been found for the vapour tension of sulphur at temperatures of 100° C. or below, but even at 100° C. its odour was most noticeable. Moreover, it was found possible to obtain a sublimation of the sulphur, the cooled end of the tube bearing numerous small needle-like crystals which gave the normal sulphur reactions.

This conclusion affords an explanation of the results of Basarow, Mach and Portele and other early investigators, who, on passing the air taken from the neighbourhood of sulphured vines through an absorbent solution found sulphate to be present after oxidation. It seems probable that the volatile sulphur derivative which they mistook for sulphur dioxide was really sulphur itself, which combining with the alkali was oxidised either by the oxidising agent used—*e.g.* chlorine—or by the air passing through the absorbent solution.

Further, a simple explanation of the formation of particulate sulphur in Barker's greenhouse experiments becomes available. Barker himself advanced no explanation of the mode of formation of particulate sulphur but indicated that under ordinary conditions in the field the process may be complex(2). In a summary (by Wilkins(33)) of the work carried out at Long Ashton on the fungicidal action of sulphur it is stated: "The older views assumed that either some poisonous gas was being given off by the sulphur, or else that sulphur itself was actually volatilising. It has been proved, however, that neither explanation is correct but that the sulphur actually discharges into the atmosphere minute particles which are dispersed by the air currents and finally reach the plant."

It would seem unnecessary, however, in view of the above experiments, to introduce any further process than the mere condensation of the sulphur vaporised from the hot-water pipes to account for the formation of the particulate sulphur removed by filtration through the cellulose pads of Barker's experiments. It appears probable that if those pads had been heated to a temperature equal or above that of the hot-water pipes the loss of fungicidal power of the filtered air would not have

been observed. In the same way, had the bent tube been similarly heated the deposition of particulate sulphur would probably not have occurred, for, in our experiments, it was found that whereas in a cold straight tube the deposition of sulphur from the heated air was rapid, in the heated tube no film of deposited sulphur was formed.

It was with the object of testing more conclusively these points that the biological trials to be described later were undertaken.

The formation of sulphur dioxide.

As the copper foil failed to detect the presence of sulphur dioxide, further experiments were conducted with this purpose in view. Difficulty was encountered however in the provision of a test sufficiently delicate for use, and after trials an unbuffered water solution of an indicator such as the B.D.H. Universal Indicator was selected as the most sensitive. Such a reagent would also respond to hydrogen sulphide, but as the lead acetate test failed to reveal the formation of this gas, it was evident that an acid reaction would indicate the formation of sulphur dioxide.

It may suffice to give the results of a series of experiments conducted more with the view of testing the findings of Atherton Lee and Martin(1) that admixture to the sulphur of an oxidising agent such as potassium permanganate resulted in an increase of fungicidal efficiency, an enhancement they considered due to a greater formation of sulphur dioxide. Two tubes (Fig. 2) were set up, in one was placed a mixture of 4 gm. of ground sulphur upon 2 gm. of glass wool, whilst the second contained 4·5 gm. of a sulphur-potassium permanganate mixture (9 parts sulphur, 1 part potassium permanganate) upon 2 gm. of glass wool. Air from outside the laboratory was drawn upwards through the tubes, first passing through potash bulbs to remove carbon dioxide and then through washing bulbs containing boiled-out distilled water. The exit air was passed through a wad of glass wool to remove solid particles and then through a dilute solution of the indicator in boiled-out distilled water.

As the efficiency of sulphur as a fungicide is increased at higher temperatures, the oxidation of the sulphur should likewise be promoted. The tubes were therefore placed in a steam bath at 100° C. throughout the day (9.0 a.m.–7.30 p.m.), the steam bath being turned out at night. Changes of colour of the indicator were noted with the results shown in Table II. Tests in which traces of sulphur dioxide were introduced into the air prior to passage through the apparatus showed a definite response in the more acid colour of the indicator.

Table II.

After (hrs)	Indicator used: Alcoholic methyl red adjusted to distinct yellow — S alone	Alcoholic methyl red adjusted to distinct yellow — S + $KMnO_4$	B.D.H. Universal Indicator adjusted to yellowish green, pH = 7·0–7·5 — S alone	B.D.H. Universal Indicator adjusted to yellowish green, pH = 7·0–7·5 — S + $KMnO_4$	B.D.H. Universal Indicator adjusted to definite greenish yellow, pH = 7·5 — S alone	B.D.H. Universal Indicator adjusted to definite greenish yellow, pH = 7·5 — S + $KMnO_4$
½	Slight reddish tint	Yellow	Slightly greener, pH = 7·5	No change	—	—
1	Yellow	,,	—	—	—	—
5	—	—	—	—	Greener in tint, pH = 7·5–8·0	Greener in tint, pH = 8·0
8	Yellow	Yellow	Slightly greener, pH = 7·5	No change	—	—
20	,, *	,, *	—	—	Definite green, pH = 8·0–8·5	Definite green, pH = 8·0–8·5
24	—	—	Original colour	No change	—	—
48	—	—	Distinct green*, pH = 8·0	Pinkish tint*, pH = 6·0	Definite green, pH = 8·0–8·5	Definite green, pH = 8·0–8·5
52	—	—	Original colour	Original colour	—	—
72	—	—	—	—	Definite green, pH = 8·0–8·5	Definite green, pH = 8·0–8·5
96	—	—	—	—	,,	,,
120	—	—	—	—	,,	,,

* These readings were taken after air had been passed rapidly through the apparatus.

That the negative result is not due to an absorption of any sulphur dioxide formed by the slight initial alkalinity of the glass wool (which was carefully washed and dried before use) was shown by this control experiment, while it may be recorded that the hot-water extract of the sulphur-glass wool mixture after the first trial run gave only a slight sulphate reaction. It is probable that this arose from the formation and oxidation, not of an alkali sulphite, but of an alkali sulphide and is an indication of the difficulty of removing completely the alkali adsorbed on the glass wool.

The results definitely indicate the non-production of a volatile sulphur derivative of acid reaction when either the sulphur-glass wool or the sulphur-potassium permanganate-glass wool mixtures are exposed to air in the presence of heat and moisture, a conclusion in accordance with the copper foil experiments. Further, they show that the addition of potassium permanganate to the sulphur does not bring about an increased formation of sulphur dioxide, a result perhaps not unexpected, for it is difficult to see why, if sulphur dioxide were formed, it would not be oxidised to sulphuric acid by the potassium permanganate.

Summary.

An examination of the various theories put forward to account for the fungicidal action of sulphur when applied, not to the plant or fungus, but to a heated surface, has been carried out by chemical methods, and it is concluded:

1. That, since the volatile agent is capable of passing a glass-wool filter maintained at the temperature of the heated surface, it is gaseous in character.

2. That the removal of the volatile agent by passage through a cooled glass-wool filter is proof that it is neither sulphur dioxide nor hydrogen sulphide but is elementary sulphur.

3. That the condensation of sulphur volatilised from the heated surface appears sufficient to account for the reactions ascribed to particulate sulphur.

REFERENCES.

(1) Atherton Lee, H. and Martin, J. P. (1928). *Indus. Eng. Chem.* xx, Jan.
(2) Barker, B. T. P. (1926). *Ann. Appl. Biol.* xiii, 313.
(3) Barker, B. T. P., Gimingham, C. T. and Wiltshire, S. P. (1919). *Ann. Rpt. Agric. Hort. Res. Sta.* Long Ashton, p. 57.
(4) Barker, B. T. P. and Wallace, T. (1921). *Ibid.* Long Ashton, p. 122.
(5) Basarow, A. (1882). *Weinlaube*, xiv, 529.
(6) Bergmann. See *Gard. Chron.* 1852, p. 419.
(7) Blodgett, F. M. (1913). *Cornell Univ. Agric. Exp. Sta. Bull.* 328.
(8) Bruck, W. F. (1912). *Plant Diseases*, trans. by J. R. Ainsworth-Davis, London p. 141.
(9) Butler, O. (1923). *New Hampshire Agric. Exp. Sta. Tech. Bull.* 22.
(10) Cockerham, K. L. (1923). *A Manual for Spraying*, New York, p. 23.
(11) Cunningham, G. H. (1925). *Fungous Diseases of Fruit-Trees in New Zealand*, Auckland, p. 71.
(12) Debus, H. (1888). *Journ. Chem. Soc.* liii, 279.
(13) Doran, W. L. (1922). *New Hampshire Agric. Exp. Sta. Tech. Bull.* 19.
(14) Eyles, F. (1926). *Rhodesia Agric. Journ.* xxiii, 929.
(15) Gimingham, C. T. (1923). *Ann. Appl. Biol.* xiii, 317.
(16) Goodwin, W. and Salmon, E. S. (1927). *Journ. Min. Agric.* xxxiv, 517.
(17) Hallock, W. (1889). *Amer. Journ. Sci.* (3) xxxvii, 405.
(18) Lees, A. H. (1923). *Journ. Pom. Hort. Sci.* iii, 103.
(19) Mach, E. and Portele, K. (1884). *Weinlaube*, xvi, 433.
(20) Mangini (1871). *Ibid.* iii, 18.
(21) Marcille, R. (1911). *Compt. Rend.* cliii, 780.
(22) Marès, H. See Prilleaux, E., *Maladies des Plantes Agricoles*, Paris, n.d. ii, 27.
(23) Marshall Ward, H. (1901). *Disease in Plants*, London, p. 161.
(24) Moissan, H. (1903). *Compt. Rend.* cxxxvii, 547.
(25) Moritz, J. (1880). *Landw. Vers. Sta.* xxv, 1.

(26) Muth, F. (1920). *Wein u. Rebe*, II, 411.
(27) Pollacci, E. (1876). *Justs Bot. Jahrb.* IV, 125.
(28) Robertson, J. (1824). *Trans. Hort. Soc. London*, V, 175.
(29) Salmon, E. S. (1921). *Journ. Min. Agric.* XXVIII, 150.
(30) —— (1926). *Ann. Appl. Biol.* XIII, 308.
(31) Sestini, F. and Mori, A. (1890). *Staz. Sper. Agrar. Ital.* XIX, 257, abst. in *Bied. Centralb.* 1891, XX, 125.
(32) Vogt, E. (1924). *Angew. Bot.* VI, 276.
(33) Wilkins, V. E. (1926). *Research and the Land*, London, p. 159.
(34) Yossifovitch, M. (1923). Abst. in *Rev. Appl. Myc.* 1924, III, 251.
(35) Young, H. C. (1922). *Ann. Missouri Bot. Gard.* XI, 403.

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SOME EXPERIMENTS WITH CALCIUM CYANIDE AS A CONTROL FOR PLANT PARASITIC NEMATODES

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(With 3 Text-figures.)

Introduction.

Accounts have been published, from time to time, of experiments conducted with the object of obtaining a control of plant parasitic nematodes by means of hydrocyanic acid gas. Sodium or potassium cyanides were used for the purpose of generating the gas in all the earlier experiments, while in recent years calcium cyanide has been used, with a certain measure of success, by several workers, for the same purpose.

In California(3) it was found that calcium cyanide drilled in furrows as the ground was ploughed, at the rate of 600 lbs. or more per acre, controlled the root knot eelworm, *Heterodera radicicola* Greef., and stimulated the growth of tomato plants. Watson(5) states that applications of 500 lbs. to 750 lbs. per acre gave promising results towards the control of the same eelworm on peach trees in Florida, while applications of 1120 lbs. per acre damaged the trees. Later the same worker(6) showed that calcium cyanide, evenly distributed at the bottom of furrows, at the rate of 1200 lbs. per acre, killed all eelworms (*Heterodera radicicola*). Thorne(4) found that calcium cyanide ploughed in to a depth of 10 in. to 12 in., at the rate of 800 lbs. to 1600 lbs. per acre, reduced to a certain extent infestations of the beet eelworm, *Heterodera schachtii* Schm., but that the plots were again severely infested in the following year. Goffart(2), carrying out pot and field experiments in Germany with the same eelworm, estimated that it would cost at least £300 per acre to obtain an efficient control by means of calcium cyanide.

It can be concluded, from the above, that heavy concentrations of hydrocyanic acid gas will, under certain conditions, destroy active

nematodes in the soil, and possibly quiescent forms and eggs also. At the same time, the probability of procuring such concentrations, at anything like a reasonable cost, under conditions met with in this country appeared to be remote, even when dealing with comparatively valuable crops such as tomato and narcissus.

The writers decided, in 1925, to undertake a number of experiments in order to obtain more definite information on the subject. Granular calcium cyanide, 40 per cent. to 50 per cent. $Ca(CN)_2$, was used in each of the experiments. This is a commercial product, marketed under the name of "Cyanogas," and sold at 1*s.* 2*d.* per lb. in 100-lb. drums. The product is simple to handle in bulk and possesses the advantage of evolving hydrocyanic acid gas over a considerable period of time in the presence of moisture.

The experiments were conducted in various localities in South Devon and in the Isles of Scilly. One was carried out in a commercial glass-house and was directed against the root knot eelworm, *Heterodera radicicola* Greef, on tomato, while the remainder were directed against the bulb eelworm, *Tylenchus dipsaci* (Kühn) Bastian, on narcissus. The soil conditions encountered in the various localities were very dissimilar and a wide range of doses was used. In each case an attempt was made to apply the substance under strictly commercial conditions and the writers' thanks are due to the growers concerned, for the keen interest which they displayed and the willingness with which they placed ground and labour at their disposal. It is proposed to describe each experiment and to include an account of the mode of procedure in some detail, as a possible guide to subsequent workers.

Experiment I. Pot experiment. *Tylenchus dipsaci* in narcissus.

As a commencement, and being likely to give earlier results, a small pot experiment was carried out at the Isles of Scilly Experimental Station. Ten 10-in. pots were filled with garden soil, of a rather light sandy nature, and heavily infested with the bulb eelworms in an active state.

Mode of infection. A number of badly infested narcissus bulbs and portions of foliage were cut into fragments not more than $\frac{1}{8}$ in. square and the fragments thoroughly intermingled with the soil of each pot, care being taken to ensure an even distribution. This operation was carried out on 24. xi. 25. Previous experience with fumigation having shown that the penetration of hydrocyanic acid gas is not good, it was assumed that it would be possible for the eelworms to escape the action

of the gas if larger portions of infested bulbs were used. The object of the experiment was to permit the gas to come into contact with the eelworms themselves and a week was therefore allowed for the eelworms to enter the soil. At the end of this time it could be assumed that the conditions were not dissimilar to those pertaining to a field from which attacked bulbs had recently been removed. On 30. xi. 25, the addition of the calcium cyanide was made and at the same time healthy bulbs were planted in the pots. The cyanide was distributed by hand at a depth of about 7½ ins. and the bulbs planted firmly, with their noses about ½ in. beneath the soil surface. Field conditions were borne in mind and the distribution and placing of the cyanide were in imitation of what might be accomplished when using this material and ploughing in bulbs in one operation.

$Ca(CN)_2$ added at rate per acre of	Varieties planted	Pot numbers
300 lbs.	Soleil d'Or	A, B
400 lbs.	Emperor	A_1, B_1
600 lbs.	Empress	A_2, B_2
800 lbs.	Berkeley	A_3, B_3
Nil (control)	Incomparabilis	A_4, B_4

It may be seen from the above table that each different rate of application was duplicated and two control pots were included. The pots were placed in a cool glasshouse and kept at an average temperature of approximately 55° F. The only variation in the after treatment was constituted by the date of first watering the two series A and B. Series A was watered immediately and series B on 2. xii. 25, three days later.

Subsequent observations. Many of the plants in series A appeared to be suffering from eelworm attack by 28. i. 26. The control, 300 lbs., and 800 lbs. pots contained plants 5 in. high, showing obvious typical lesions on the leaves. A few of the bulbs were lifted and were examined in the laboratory, where the presence of eelworms in the leaves and necks of the bulbs was confirmed, but none was found elsewhere in the bulbs. At this time the bulb scales were not discoloured or in a condition, as far as the eye could discern, to lead one to suspect the presence of eelworms. Series B did not show visible signs of eelworm attack at this time. Later the disease spread rapidly in both series and before the end of the growing season all the plants were distorted and crippled.

Remarks. The above experiment, while of insufficient size to afford conclusive evidence, suggests that the application of commercial calcium cyanide in quantities up to 800 lbs. per acre is useless as a control for

bulb eelworm. At the same time, the variation in behaviour of the plants in series A and B, while in accordance with anticipations, was of interest. The principal causes of this variation are probably three in number. Atmospheric moisture is sufficient for the evolution of hydrocyanic acid gas from calcium cyanide, and it is further well known that moisture will absorb the gas. Furthermore, Campbell(1) states that when too much moisture is present the calcium cyanide breaks down, forming ammonia, and thus lessening the amount of hydrocyanic acid gas given off. Finally, eelworms find a high water-content of the soil more congenial and their freedom of movement is likely to be increased by this condition.

Experiment II. Field experiment. *Tylenchus dipsaci* in narcissus.

This experiment, carried out at Combe-in-Teignhead, South Devon, consisted of treating with calcium cyanide a portion of ground heavily infested with bulb eelworms and then planting back clean narcissus bulbs. The soil in this and the next experiment was a medium loam overlying Devonian sandstone. A bed was selected which had contained bulbs infested with eelworms for two seasons. The bulbs were lifted and the area was carefully dug over, every possible portion of bulb being removed. Calcium cyanide was then sown by hand in drills 5 in. deep and 1 ft. apart and covered immediately. Four drills were sown, each being 45 yds. long, giving a treated area of 1/200 of an acre. The cyanide was applied on 10. ix. 25, 7 lbs. being used, giving a dose of 1400 lbs. per acre. An equal area of ground was reserved adjacent to the treated area to serve as a control and was dug in the same manner.

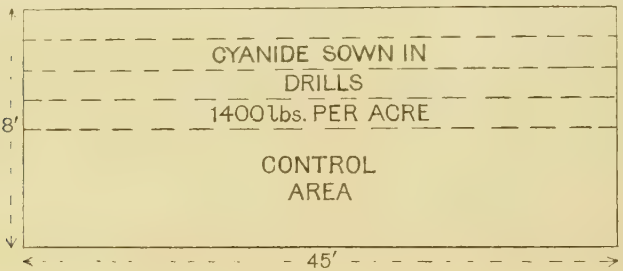


Fig. 1.

Fourteen days after the application of the cyanide, bulbs, known to be free from eelworms, were planted over the whole bed. The position

of the cyanide drills had been denoted by stakes and the bulbs were planted in rows running at right angles to the former.

Subsequent observations. The bed was kept under constant observation and foliage first appeared above ground on 3. xii. 25. By the end of January, 1926, foliage apparently attacked by eelworms was observed in both treated and untreated areas. Laboratory examination showed eelworms to be abundant in material collected from both plots. No difference in the degree of infestation in the two areas was visible during the season 1926 and the bulbs were left in the ground. Early in 1927 an inspection was made, and so heavy had eelworm damage become that 60 per cent. of the bulbs had failed entirely. Again no difference between the two plots could be found and the experiment had to be abandoned as the ground was required for other purposes.

Remarks. The complete failure of a dose of 1400 lbs. of calcium cyanide per acre to produce appreciable control of the eelworms was disappointing. It is admittedly possible, or even probable, that eelworm invasion of the treated area may have occurred by 1927. At the same time, numerous field observations have been made regarding the normal rate of spread of eelworms from bulb to bulb. These indicate the extreme improbability of such invasion being responsible for the initial heavy infestation of the bulbs throughout the treated area, only four months after the treatment had been made.

Experiment III. Field experiment. *Tylenchus dipsaci* in narcissus.

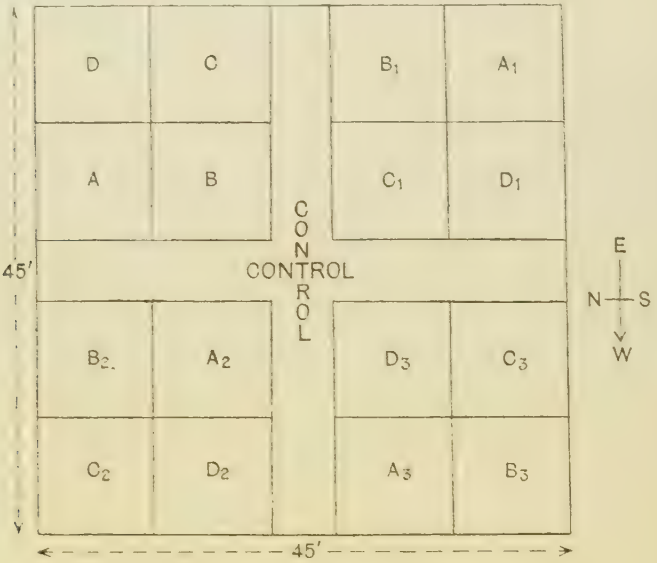
This experiment, in reality an elaboration of the preceding one, was carried out in the same locality. A large bed was selected, which had contained eelworm infested bulbs for three years. The bulbs were removed and the ground dug as previously. Calcium cyanide was sown by hand, in drills 6 in. deep and 18 in. apart and covered immediately. It was decided to compare the effect of various doses and the cyanide was therefore used at the rates of 750, 1000, 1500 and 2000 lbs. per acre. Sixteen plots were treated, each being 1/400 of an acre in extent. Each dose was repeated on four plots and an ample central control area was left. The application was made on 2. x. 25 and eelworm-free bulbs were planted over the whole sixteen days later.

Subsequent observations. The experiment was observed at intervals of approximately one month from the date of commencement in October, 1925, until March, 1927, when it was abandoned. For our present purpose

it will only be necessary to record details of observations made on three of these occasions.

5. iv. 26. Extensive eelworm damage obvious in the control plot and plots A, A_1, A_2, A_3, B, B_2, B_3 and C_3.

12. ix. 26. Twelve bulbs lifted at random from each plot and examined for eelworms. Eelworms found in control plot and plots A, A_1, A_2, A_3, B, B_1, B_2, B_3, C_2, C_3 and D_3. Eelworms absent in plots C, C_1, D, D_1, D_2.



Plots A, A_1, A_2, A_3. 750 lbs. calcium cyanide per acre.
„ B, B_1, B_2, B_3. 1000 lbs. „ „
„ C, C_1, C_2, C_3. 1500 lbs. „ „
„ D, D_1, D_2, D_3. 2000 lbs. „ „

Fig. 2.

18. iii. 27. By this date 80 per cent. of all the bulbs in the control plot had been entirely destroyed, as had 60 per cent. of those in the series of plots A and B. The ground was required and it became necessary to abandon the experiment, so 24 bulbs were taken from each plot and examined in the laboratory. Eelworms were found in bulbs from every plot excepting C, D and D_1.

Remarks. A consideration of the above data indicates that, while doses of from 750 to 1000 lbs. of calcium cyanide per acre served slightly

to reduce the eelworm population in the soil, doses of from 1500 to 2000 lbs. produced complete control in a certain number of plots. As was the case in the preceding experiment, the fact that the observations extended over a number of seasons introduces the possibility of eelworm migration among the plots. Such migration probably took place extensively, at least during the latter portion of the time, owing to the scarcity of bulbs in some plots. The experiment was conducted on a sloping piece of ground and the western and lower end was considerably damper than the eastern one. A high moisture content would tend to facilitate the movement of eelworms through the soil, and the fact that the western-most plots, which received heavy doses of cyanide, were nevertheless infested with eelworms at the close of the experiment is probably explainable in this manner.

Experiment IV. Field experiment. *Tylenchus dipsaci* in narcissus.

This experiment was carried out on the Isles of Scilly and was commenced in 1925. A portion of light and shallow land was cleared of eelworm infested narcissus bulbs, as thoroughly as possible, and calcium cyanide was drilled in at rates varying from 400 to 800 lbs. per acre. The application of the cyanide was made at the time of planting bulbs, known to be free from eelworms, over the whole area.

No sign of eelworm damage to the bulbs was observed in either treated or control plots until 9. iv. 27, when a very little was found. Under these circumstances the experiment cannot be considered of value for the purpose under consideration.

Experiment V. Glasshouse experiment. *Heterodera radicicola* in tomato.

The experiment was conducted in a commercial glasshouse near Plymouth. The house selected for the purpose had been used exclusively for tomatoes for a number of years and considerable losses, attributable to *Heterodera radicicola*, were regularly sustained. The dimensions of the house were 300 ft. by 40 ft., and it was decided to treat plots at each end of the house, leaving the remainder as a control area.

Fourteen plots, varying in area from 170 to 300 sq. ft., were treated with calcium cyanide, in doses ranging from 1000 to 2000 lbs. per acre. The limits of the plots were conveniently marked by the hot-water pipes running the length of the house. The cyanide was applied in the granular form as in the other experiments, the ground in this case being double trenched and the cyanide broadcast immediately prior to turning in the top spit. Ordinary nursery hands were employed to carry out the digging, and it may be of use to record that 7 men took 12 hours to double

trench the treated area of 340 sq. yds. An eighth man was required to superintend and to weigh out and distribute the cyanide. The immediate covering of the cyanide precluded the occurrence of strong fumes of hydrocyanic acid gas in the house and no complaints were made by the men. The work was carried out on January 13th and 14th, 1926, and the house was planted with tomatoes about two weeks later.

Subsequent observations. The first examination of the plants was made on 5. v. 26, when nodules containing eelworms were found on plants in both treated and untreated areas. Owing to the house being a commercial one it was not possible to disturb the roots of large numbers of plants during the cropping season. Observations were however made

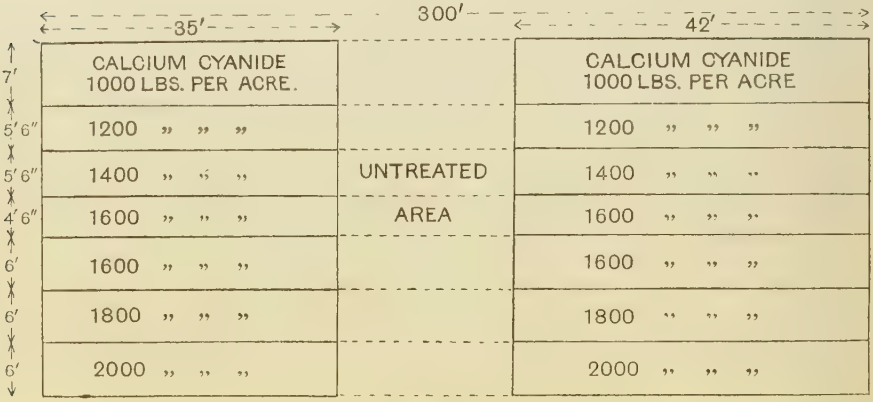


Fig. 3.

at frequent intervals and no difference in vigour or cropping could be discerned between plants in treated and untreated areas. Towards the end of the season it was possible to uproot numbers of the plants. Very heavy eelworm infestations were found throughout the treated areas and in no case were these less heavy than in the control area.

Remarks. The fact that this experiment was carried out under glass permitted a reasonable control of the soil humidity at the time of distributing the cyanide. Care was taken to avoid the presence of excessive moisture in the soil, and as a result the smell of hydrocyanic acid gas was found to persist in the soil for several days. In previous experiments carried out in the open no smell could be detected in the soil 24 hours after application.

Conclusions.

The above experiments, with the exception of No. 4, which failed owing to some reason at present not fully understood, tend to indicate that the use of granular calcium cyanide as a soil fumigant against plant parasitic nematodes is impracticable in this country.

Previous workers (*loc. cit.*) have confined their attentions to nematodes of the genus *Heterodera* and have met with varying success. The experiment with *H. radicicola* on tomato was carried out under conditions normal to tomato growing in this country. The complete failure to obtain control of the nematode, or even an appreciable reduction in its numbers, with a dose of 2000 lbs. of calcium cyanide per acre, holds out little hope of ultimate success in combating the nematode by means of this substance.

The experiments with *Tylenchus dipsaci* are perhaps of greater interest, as little or no information has so far been available on this score. The initial failure to obtain control in the pot experiment was disappointing, in that one would expect heavier doses to be necessary when working on a field scale. The failure of a dose of 800 lbs. per acre administered in a pot suggested but small likelihood of a similar dose being satisfactory in the field.

In Experiment II a dose of 1400 lbs. per acre produced no appreciable reduction of the eelworm population, while in Experiment III, of 8 plots receiving 1500 lbs. or more per acre, 5 only were free from attack at the end of the first season and 3 at the commencement of the second. The almost complete failure of the field experiments was in all probability due to the impossibility of removing from the soil all portions of the previously attacked crop. These remnants might well harbour the eelworms in safety until the effect of the cyanide had passed off, except perhaps in cases where very heavy doses were administered, when the hydrocyanic acid gas might be expected to subsist for a longer period with consequent greater penetration.

In the opinion of the writers the successful use of calcium cyanide as a soil fumigant against plant parasitic nematodes is not possible under conditions pertaining in this country. At the same time, where expense is no consideration, small areas of soil may satisfactorily be rid of nematodes by the application of very heavy doses of calcium cyanide.

SUMMARY.

1. Considerable diversity of opinion has existed regarding the practicability of controlling plant parasitic nematodes by means of granular calcium cyanide.

2. Previous experiments with this substance have invariably been directed against eelworms of the genus *Heterodera*.

3. The experiments described in this paper were carried out in South Devon and in the Isles of Scilly and with the exception of one with *Heterodera radicicola*, in tomato, relate to *Tylenchus dipsaci*, in narcissus.

4. Doses of granular calcium cyanide, ranging from 300 lbs. to 2000 lbs. per acre, were employed under various conditions. Only with doses of from 1500 lbs. to 2000 lbs. per acre was any real indication of satisfactory control of *T. dipsaci* obtained, while *H. radicicola* remained unaffected by similar doses.

5. In the opinion of the writers the successful use of granular calcium cyanide for the control of plant parasitic nematodes, on a commercial scale, is not possible under conditions pertaining to this country. At the same time, where expense is of no account, small areas of soil may be rid of nematodes by means of exceedingly heavy doses of the substance.

REFERENCES.

(1) CAMPBELL, R. E. (1924). Preliminary Report on the Use of Calcium Cyanide as a Soil Fumigant for Wireworms. *Journ. Econ. Ent.* XVII, no. 5, 562–7. Geneva, N.Y., October.

(2) GOFFART, H. (1927). Versuche zur Bekämpfung der Rübennematoden (Heterodera schachtii, Schm.) mit Calciumcyanid. *Arb. biol. Reichsanst. Land- u. Forstw.* XV, no. 2, 249–59, 16 refs. Berlin, February.

(3) (1926). Entomological Work of the Year. *Rept. California Agric. Expt. Sta.* 1924–5, pp. 43–6 and 51–3. Berkeley, Cal.

(4) THORNE, G. (1926). Control of Sugar-beet Nematode by Crop Rotation. *U.S. Dept. Agr. Farmers' Bull.* 1514, 20 pp., 15 figs. Washington, D.C., November.

(5) WATSON, J. R. (1925). Report of Entomologist. *Florida Agr. Expt. Sta. Rept.* 1924–5, pp. 36R–39R. Gainesville, Fla.

(6) —— (1925). Mole-crickets. *Florida Agr. Expt. Sta., Press Bull.* 372, 2 pp. Gainesville, Fla., September.

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LABORATORY EXPERIMENTS WITH NON-ARSENICAL INSECTICIDES FOR BITING INSECTS

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(With Plate XXXVII.)

The use of fluids or dusts containing arsenic compounds for the control of biting insects of economic importance is attended by certain obvious disadvantages and a good deal of work has been done in recent years in the attempt to find satisfactory substitutes for "arsenicals." In an interesting paper(1) Moore and Campbell give a brief account of preliminary laboratory experiments with a considerable number of compounds, both organic and inorganic, as stomach poisons. The work had specially in view the control of the Japanese beetle (*Popillia japonica*), but other beetles and Tent caterpillars were also used. The insects, usually 20 in number, were placed in a cage, and sprayed or dusted foliage of either potted plants or freshly cut shoots in water was then introduced. The number of insects dead at the end of each 24-hour period was recorded, together with observations on the amount of feeding and on injury to the foliage, if any. The results were chiefly of theoretical interest, copper cyanide being the only non-arsenical tested which had a toxicity to the Japanese beetle comparable with that of lead arsenate. It caused no damage to foliage. Copper thiocyanate showed high toxicity to Tent caterpillars but was non-toxic to Japanese beetle. Hargreaves(2) has also tested a number of miscellaneous organic compounds. Emulsions of the various substances with a liquid soap were prepared, and after immersing leaves in 90 per cent. alcohol and allowing them to become nearly dry, the mixtures were applied with a brush. Larvae of *Pieris rapae* on cabbage and *Spilosoma lubricipeda* on lupins (5 larvae per test) were used, but many of the experiments were complicated by the fact that some of the larvae were parasitised. Salts of the dinitro-cresols, some naphthalene derivatives and barium and calcium fluorides are

recorded as having high toxicity. No reference is made to the effect of the chemicals on the foliage. The dinitro-cresylates are known to be highly injurious to many kinds of foliage (3) and therefore, although very toxic, they are not likely to be of practical interest as possible ingredients of spray-fluids for summer use.

Among possible substitutes for arsenicals, perhaps most attention has been paid to compounds containing fluorine. The toxicity of sodium fluoride to cockroaches was investigated by Shafer (4) and it is frequently used in poison baits for these insects; more recently its possibilities as a locust poison have been pointed out by Ripley (5), who found that baits of grass or fresh horse dung moistened with a 2 per cent. solution of sodium fluoride or dusted with a 1 : 50 mixture of sodium fluoride and lime proved highly effective. It is less poisonous but also less repellent[1] to the insects than sodium arsenate. Unfortunately, sodium fluoride solutions, even when very dilute, cause serious damage to foliage and are therefore not suitable for use against leaf-eating insects in the form of summer spray-fluids. This objection is not shared to the same extent by certain silicofluorides. These compounds have long been known to have insecticidal and fungicidal properties, and Marcovitch has recently published a number of papers (6) on the efficiency of sodium and calcium silicofluorides, when used in the form of dusts, as insecticides for biting insects. Sodium silicofluoride mixed with 9 parts of hydrated lime gave good control against the Mexican bean beetle (*Epilachna corrupta*) and other insects and can also be used alone, at all events on certain plants. Calcium silicofluoride is similarly effective, but when undiluted causes severe foliage injury; it can however apparently be safely used mixed with lime or other diluents, and dusting preparations of this material are now on the market.

Roark (7) has pointed out that commercial grades of sodium silicofluoride may contain some sodium carbonate, and that in the presence of water the silicofluoride would be decomposed by the alkali and sodium fluoride formed. This would cause injury to foliage. Similarly, if mixed with hydrated lime, sodium silicofluoride would be converted into calcium silicofluoride and then into calcium fluoride. Alkaline water or alkaline exudations from the plants to which the material is applied might also bring about formation of fluorides. Roark suggests that the formation of fluorides accounts, at all events to some extent, for the insecticidal action of silicofluorides and that the use of some of the less

[1] H. W. Thompson has, however, found that a sodium fluoride poison bait is of little use against "leatherjackets." (*Welsh Journ. Agr.* 1926, II, 228.)

soluble fluorides which are not injurious to foliage would result in more uniform insecticidal action. Marcovitch however maintains that the silicofluorides are more effective than relatively insoluble fluorides and that they can be safely used.

More recently a report has appeared from the United States Chemical Warfare Service Cotton Boll Weevil Investigation, by Walker and Mills(8), which gives an account of laboratory and field experiments with a large number of substances tested as dusts against the cotton boll weevil (*Anthonomus grandis*). Ordinary commercial sodium silicofluoride was found more toxic than commercial calcium arsenate volume for volume, but owing to its greater density two to four times the weight was required to cover the same area. A special form of sodium silicofluoride was however prepared, having a much lower density, which was found to be as effective weight for weight as calcium arsenate and which caused only negligible injury to cotton. A specially prepared barium silicofluoride was similarly effective. Both compounds were superior to calcium arsenate in adhesive power. Further field experiments are planned.

Langford(9) and other workers in America have successfully used sodium silicofluoride as a poison in baits for grasshoppers and other insects.

Quantitative data in regard to the efficiency of stomach poisons are not easy to obtain. Some interesting work in this direction has been done recently by Campbell(10) who has based a quantitative method on the observation that certain caterpillars will absorb completely drops of liquid placed on the leaf in their feeding path, if the liquid is not too distasteful. By suitable manipulation individual larvae can thus be given known doses of toxic substances, and the times of survival noted. A quantitative basis for "comparison of susceptibilities" can thus be arrived at. The silkworm is considered to be specially suitable for this type of work. The results so far reported deal with arsenic compounds, and it has been shown by this method that the susceptibility of silkworms (*i.e.* the effects of equal doses per unit weight of animal) to arsenic decreases during larval development. Campbell has also used his technique to investigate the interesting question of the possible development of tolerance to arsenic, and preliminary experiments show that individual tolerance to arsenic was not induced in silkworms by quantitative feeding of sublethal doses of sodium arsenate solution.

Janisch(11) has described a method which aims at obtaining quantitative data by weighing the poisoned leaves and tracing their outlines

on squared paper before and after the insects have fed on them and thus ascertaining the quantity of poison ingested.

Van Leeuwen (12), working on the toxicity of lead arsenate to the Japanese beetle (*Popillia japonica*), measured the area of treated foliage consumed and the quantity of arsenic causing death; and Newcomer (13) has described a technique suitable for use in laboratory experiments on the toxicity of stomach poisons to Codling moth larvae.

A detailed account has also been published by Kalandadze (14) of experiments with arsenical dusts on several insects which are pests of forest trees. Large glass cylindrical jars were used as cages with the foliage in water in a small bottle with cotton-wool plug. In each series of experiments the same amount of foliage was used in each cage, leaves or shoots being counted, and the amount eaten at the end of the experiment determined, either as surface area or as number of leaves or needles. Larvae of *Lymantria dispar*, *L. monacha* and *Bupalus piniarius* were tested at each instar and were allowed to complete development if possible. The arsenic content of some of the poisoned insects was determined, and the excreta in each cage collected and weighed at the end of the experiment. Among other results it was found that the minimum toxic dose of arsenic increased with the age of the larvae and that freshly moulted individuals are more susceptible than those which have moulted some time. Partially poisoned larvae often recovered when given fresh foliage, but such recovery was sometimes only apparent, the larvae failing to complete development. In the case of larvae which received a sublethal dose of arsenic and did succeed in completing development, there appeared to be an "after-effect" of the poison since eggs laid by adults arising from such larvae almost always failed to hatch.

In the past few years the writers have made laboratory experiments on the toxicity of various substances as stomach poisons to biting insects, when applied to foliage as sprays. A simple technique is employed for these tests, and although it does not differ in principle from methods adopted by other workers a short description may be of interest.

Hurricane lamp glasses are used as the separate cages required for each test. They are fitted with bungs at the smaller end, each bung having a hole bored through the middle. Shoots of the food plant of convenient size are cut and the foliage stripped off at the lower end so as to give a long stalk. The foliage is sprayed, by means of a small bottle sprayer, with the liquids to be tested and the shoots suspended head downwards until dry; each is then placed in a lamp glass, the stalk being pushed

through the hole in the cork. The glasses are supported in any suitable manner with the stalks dipping into water; wooden trays with spaces between the slats are convenient, with a separate test-tube below for each stalk. With plants such as hawthorn, hazel, black currant, etc., the foliage keeps fresh for 10 days or longer, if the water is renewed as required. A counted number of caterpillars or other insects are placed in each cage and the top covered with muslin attached to an iron ring, which holds it in place and avoids the need for tying[1]. When very young larvae are used, a tight fit where the stalk of the shoot passes through the cork is ensured by packing with a little cotton wool. Fig. 1 shows a single cage containing a shoot of hawthorn, supported on a tripod, and Fig. 2 a dozen arranged on a tray. With cages of this type a considerable number of tests can be run concurrently without much trouble and at no great expense, and it is a simple matter to examine the insects at intervals, and to observe the extent of feeding and the effect of the spray-fluid on the foliage. At each examination the number of insects "unaffected," "slightly affected," "moribund" and "dead" are recorded; and in the case of larvae notes are also made in regard to their growth.

It is advisable to carry on experiments of this type for not less than 8 or 10 days before drawing definite conclusions. It has been observed by Krasilschtschik (15) that in many cases the maximum mortality is not reached until about the eighth day; and this has also been our experience. Except in cases where the substance used renders the foliage very repellent, larvae which survive beyond the eighth day are usually capable of completing their development. Several species of lepidopterous larvae, including *Selenia tetralunaria*, *Orgyia antiqua*, *Abraxas grossulariata*, and *Cheimatobia brumata* have been used, stocks being reared under as natural conditions as possible, but protected from the attacks of parasites.

It is not proposed to discuss the results obtained in any detail because there are a number of irregularities and discrepancies which will need much further work before they can be cleared up. A brief reference to the kind of results obtained with two dissimilar groups of compounds may, however, serve a useful purpose.

The silicofluorides[2] are perhaps of special interest since, as already mentioned, they have been recommended for use as the toxic ingredient

[1] The writers are indebted to Mr E. E. Green, through Dr A. D. Imms, for this convenient means of covering small cages.

[2] A note on some early experiments with silicofluorides was published in *Ind. Eng. Chem.* 1925, xvii, 323.

Table I.

Silicofluorides.

Substance	Concentration in gm. per 100 c.c.	Larvae	% not affected	% dead	Foliage	Injury to foliage	Date of Exp.
Sodium silicofluoride	1·0 0·5	C. brumata	—	100	Apple	Severe	25. v.
	0·25	,,	—	80	,,	,,	,,
	1·0 & 0·75	S. tetralunaria (young)	—	100	Hawthorn	Variable:	29. v.
	0·5 & 0·25	,,	—	100	,,	not great	,,
	0·5	S. tetralunaria (older)	—	100	,,	None	17. vi.
	0·25	,,	—	40	,,	Slight	,,
	0·1	,,	100	—	,,	None	,,
	0·5	S. tetralunaria (young)	—	100	,,	Considerable	15. viii.
	0·25	,,	—	80	,,	Slight	,,
	0·1	,,	100	—	,,	Traces	,,
	0·5	A. grossulariata	—	40	Black currant	Some	17. viii.
	0·25	,,	—	23	,,	Traces	,,
	0·1	,,	100	—	,,	None	,,
Potassium silicofluoride	1·0–0·5	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
	0·25	,,	—	80	,,	,,	,,
	0·5	S. tetralunaria (older)	—	30	,,	,,	17. vi.
	0·25	,,	30	10	,,	,,	,,
	0·1	,,	100	—	,,	,,	,,
	0·5	S. tetralunaria (young)	—	80	,,	Severe	15. viii.
	0·25	,,	—	60	,,	Slight	,,
	0·5	A. grossulariata	—	60	Black currant	None	17. viii.
	0·25	,,	—	40	,,	,,	,,
	0·1	,,	80	20	,,	,,	,,
Aluminium silicofluoride (B.D.H.)	1·0	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
	0·75	,,	—	55	,,	,,	,,
	0·5	,,	—	40	,,	,,	,,
	0·25	,,	50	30	,,	,,	,,
	1·0	S. tetralunaria (older)	—	60	,,	,,	17. vi.
	0·75	,,	—	70	,,	,,	,,
	0·5	,,	10	40	,,	,,	,,
	0·25	,,	100	—	,,	,,	,,
	1·0	S. tetralunaria (young)	—	80	,,	Slight	15. viii.
	0·5	,,	—	40	,,	Severe	,,
	0·25	,,	—	—	,,	,,	,,
	1·0	A. grossulariata	80	—	Black currant	None	17. viii.
	0·5	,,	80	—	,,	Very slight	,,
	0·25	,,	80	—	,,	None	,,
Aluminium* silicofluoride	1·0–0·75	S. tetralunaria (older)	—	100	Hawthorn	Traces	17. vi.
	0·5	,,	—	60	,,	,,	,,
	0·25	,,	—	50	,,	Slight	,,
	0·5	S. tetralunaria (young)	50	30	,,	Severe	15. viii.
	0·25	,,	30	20	,,	,,	,,
	0·1	,,	50	—	,,	Considerable	,,
	0·5	A. grossulariata	—	30	Black currant	,,	17. viii.
	0·25	,,	50	—	,,	Very slight	,,
	0·1	,,	50	—	,,	Traces	,,
Calcium silicofluoride	1·0–0·75	S. tetralunaria (young)	—	100	Hawthorn	Irregular	29. v.
	0·5	,,	—	100	,,	Very slight	,,
	0·25	,,	50	50	,,	,,	,,
	1·0–0·75	S. tetralunaria (older)	—	100	,,	Considerable	17. vi.
	0·5	,,	—	20	,,	? traces	,,
	0·25	,,	—	40	,,	Some	,,
	1·0	S. tetralunaria (young)	100	—	,,	Severe	15. viii.
	1·0	A. grossulariata	100	—	Black currant	Some	17. viii.
Lead Arsenate	1·0–0·5	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
Controls		,,	100	—	,,	—	,,
		,,	100	—	,,	—	15. viii.
		S. tetralunaria (older)	95	5	,,	—	17. vi.
		A. grossulariata	100	—	Black currant	—	17. viii.

* Precipitated from aluminium sulphate using a slight excess of sodium silicofluoride.

of dusting preparations. Used as spray-fluids in solution or suspension in a 1 per cent. solution of saponin, we have found the silicofluorides of sodium, potassium, calcium and aluminium to have considerable toxicity as stomach poisons to young larvae of several species of moths; but the power of resistance varies with the different species and is markedly greater with older larvae. In regard to injury to foliage the results were extremely variable and difficult to interpret; the extent of injury not only differed with different plants but also with the same plant on different occasions. The observations indicate that, with hawthorn, the foliage is more sensitive to injury by silicofluorides in the latter part of the summer than in May or June.

Table I gives some examples of the kind of data obtained.

It is evident that, in spite of the considerable toxicity of various silicofluorides, they cannot be suggested at the present stage even for larger scale field experiments as sprays for use on foliage. There are some conditions under which these compounds appear to cause little or no damage to foliage, but a much more extensive series of laboratory experiments than it has been possible to make at present is required to establish these conditions.

Results of a quite different kind were given by the use of extracts of certain tropical leguminous plants known to have a high toxicity as contact insecticides (16). In this case the question of risk of injury to foliage does not arise; the extracts are quite harmless to plants. The outstanding fact shown by the experiments with these materials was their extremely repellent action. Foliage sprayed with extracts of these plants even at high dilutions remained untouched by the larvae in almost every case; rather than eat it, the larvae eventually died of starvation. Details of some tests with *Tephrosia vogelii*, *T. toxicaria* and *T. macropoda*, and with Black and White Haiari (species of *Lonchocarpus* from British Guiana) are given in Table II.

The possible practical value of repellents in combating leaf-eating insects has been little worked on; it might be considerable in special cases and would seem worth following up.

Results from the type of experiments discussed indicate a marked degree of specificity in the resistance of insects to stomach poisons and in the action of different substances upon foliage. They call for a more quantitative investigation of the whole subject.

F. L. Campbell (17) has recently put forward a plea for the development of laboratory research on the effects of poisons on insects on strictly quantitative lines, and his work on quantitative methods for the in-

Table II.

Extracts of some tropical plants.

(N = not affected: S = slightly affected: M = moribund: D = dead.)

Extract of	Concentration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
Tephrosia	2·0	O. antiqua (half grown)	—	90	—	10	Practically none: starved
Vogelii	1·0	,,	—	90	—	10	,, ,,
(leaves)	0·75	,,	—	70	20	10	,, ,,
	0·5	,,	10	90	—	—	Very little: slight growth
Black	1·0	,,	—	20	60	20	Practically none: starved
Haiari	0·75	,,	—	80	20	—	Very little: starved
(stems)	0·5	,,	—	80	20	—	,, ,,
	0·25	,,	10	50	40	—	Considerable
Black	1·0	,,	—	70	30	—	Practically none: starved
Haiari	0·75	,,	—	100	—	—	,, ,,
(roots)	0·5	,,	—	80	20	—	,, ,,
	0·25	,,	20	60	20	—	Considerable
White	1·0	,,	20	80	—	—	Evident, but less than normal
Haiari	0·75	,,	30	70	—	—	,, ,,
(stems)	0·5	,,	50	50	—	—	,, ,,
	0·25	,,	100	—	—	—	Normal
White	1·0	,,	—	90	10	—	Very little: starved
Haiari	0·75	,,	—	80	—	20	,, ,,
(roots)	0·5	,,	—	100	—	—	Little
	0·25	,,	70	30	—	—	Considerable
Controls	—	,,	100	—	—	—	Normal
	—	,,	100	—	—	—	,,
	—	,,	100	—	—	—	,,
Tephrosia	2·0	S. tetralunaria (young)	—	—	—	100	None: starved
Vogelii	1·0	,,	—	—	—	100	,,
(leaves)	0·5	,,	—	—	20	80	,,
	0·25	,,	—	100	—	—	Evident, but less than normal
Black	2·0	,,	—	—	—	100	None: starved
Haiari	1·0	,,	—	—	—	100	,,
(stems)	0·5	,,	—	—	50	50	,,
	0·25	,,	—	90	—	10	Some: slight growth
Black	1·0	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	Very little: starved
(roots)	0·25	,,	—	60	—	40	,, ,,
White	1·0	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	,,
(stems)	0·25	,,	—	80	—	20	Very little: starved
White	1·0	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	,,
(roots)	0·25	,,	—	—	40	60	,,
Controls	0·25 % soap	,,	100	—	—	—	Normal: good growth
	,,	,,	100	—	—	—	,, ,,
	Unsprayed	,,	100	—	—	—	,,

Table II (*continued*).

(N = not affected: S = slightly affected: M = moribund: D = dead.)

Extract of	Concentration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
White Haiari (stems)	1·0	C. brumata (young)	—	—	—	100	None*
	0·5	,,	—	—	—	100	
White Haiari (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Black Haiari (stems)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Black Haiari (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Tephrosia Vogelii (leaves)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Tephrosia Toxicaria (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Tephrosia Macropoda (stems)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	10	90	
Tephrosia Macropoda (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Controls	0·25 % soap	,,	100	—	—	—	Normal: larvae pupated
	Unsprayed	,,	100	—	—	—	,, ,,
Black Haiari (stems)	1·0	T. gothica (10–14 days old)	—	100	—	—	Appreciable feeding but very little growth
	0·5	,, ,,	—	100	—	—	Appreciable feeding and some growth, but less than normal
Controls	0·25 % soap	,, ,,	100	—	—	—	Normal
	Unsprayed	,, ,,	100	—	—	—	,,

* Results were identical on apple and hawthorn. Strong repellent action in all cases so that larvae died of starvation. 0·25 % soap was used with all the extracts.

vestigation of stomach poisons has already been referred to. We have long been convinced that the quantitative method of approach to the problems of insecticides and insecticidal action is the only one likely to lead to further advances of economic importance, and we have for some years been engaged on the study of contact insecticides from a quantitative point of view. Our experience in regard to work with stomach poisons is less extensive, but the results discussed serve to emphasize the need for a knowledge of insect toxicology (to use Campbell's term) on a systematic quantitative basis.

SUMMARY.

1. A convenient technique for experiments with insecticides for biting insects is described.

2. The silicofluorides of sodium, potassium, aluminium and calcium, used in the form of spray-fluids, showed considerable toxicity to young larvae of several species of moths. The degree of resistance varies with different species and is greater with older larvae. Considerable, but irregular, injury to foliage was noted, and much further work is required to establish the conditions under which these compounds could be safely used.

3. Foliage sprayed with extracts of certain tropical plants is extremely repellent to young larvae. Even with high dilutions of the extracts, the foliage remained uneaten and the larvae eventually died of starvation.

4. A short review of some recent work on laboratory experiments with non-arsenical insecticides for biting insects is given.

LITERATURE CITED.

(1) MOORE, W. and CAMPBELL, F. L. (1924). *Journ. Agr. Res.* XXVIII, 395.

(2) HARGREAVES, E. (1924). *Bull. Ent. Res.* XV, 51.

(3) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* XII, 218.

(4) SHAFER, G. D. (1916). *Mich. Agr. Coll. Expt. Sta., Tech. Bull.* 21.

(5) RIPLEY, L. B. (1924). *Bull. Ent. Res.* XV, 29.

(6) MARCOVITCH, S. (1924). *Tenn. Agr. Expt. Sta. Bull.* 131; 1924, *Ind. Eng. Chem.* XVI, 1249; 1925, *Science*, LXI, 22; 1925, *Journ. Econ. Ent.* XVIII, 122; 1926, *Tenn. Agr. Expt. Sta. Bull.* 134; 1926, *Journ. Econ. Ent.* XIX, 795; 1926, *Science*, LXIV, 159.

(7) ROARK, R. C. (1926). *Science*, LXIII, 431.

(8) WALKER, H. W. and MILLS, J. E. (1927). *Ind. Eng. Chem.* XIX, 703.

(9) LANGFORD, G. S. (1926). *Journ. Econ. Ent.* XIX, 670.

(10) CAMPBELL, F. L. (1926). *Journ. Agr. Res.* XXXII, 359; *Journ. Econ. Ent.* 1926, XIX, 516; *Journ. Gen. Physiol.* 1926, IX, 433, 727.

(11) JANISCH, R. (1926). *Nachrichtenbl. deut. Pflanzenschutzdienst*, VI, 18.

(12) LEEUWEN, E. R. VAN (1927). *Journ. Agr. Res.* XXXIV, 1043.

(13) NEWCOMER, E. J. (1926). *Ibid.* XXXIII, 317.

(14) KALANDADZE, L. (1927). *Zeit. Angew. Ent.* XIII, 1.

(15) KRASILSCHTSCHIK, J. M. (1903). *Arb. Ent. Bureau, St Petersburg*, IV (Abstract in *Z. wiss. Insektenbiol.* 1910, VI, 120).
Cf. also BARSACQ, J. (1907). *Rev. Sci.* VII, 721.

(16) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* XII, 61; 1926, XIII, 424.

(17) CAMPBELL, F. L. (1926). *Science*, LXIII, 45.

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Fig. 1. Apparatus used for experiments with stomach poisons: a single cage.

Fig. 2. Apparatus used for experiments with stomach poisons.

THE TURNIP MUD BEETLES (*HELOPHORUS RUGOSUS* OL. AND *HELOPHORUS PORCULUS* BEDEL.)

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(With Plate XXXVIII and 8 Text-figures.)

CONTENTS.

The first record of damage by *Helophorus rugosus* in Great Britain was made by Miss Ormerod[1] in which she states that "On June 25th, 1899, Mr John Milne of Inverurie, Aberdeenshire, forwarded specimens of the beetle with the information that they were destructive to the turnip crop in its early stages." As the beetles are usually covered with soil she suggests the name "Turnip Mud-beetle" as conveniently describing the mixed nature of its habits, or places of resort. Mr Milne further states "I have observed turnip fields attacked at the side next a former turnip field, here and there, throughout this part of the country for over thirty years. The mischief is done when the plants are small."

On September 28th, 1894, and again early in November, Miss Ormerod received turnips from Aberdeenshire with larvae feeding in the upper

parts of the "bulbs" and the leaf stalks and also with adults feeding on the leaves. On September 5th, 1895, she received further specimens with larvae injuring the leaves.

She also states "The earliest date of receiving these beetles was as near as may be June 26th, but they had been watched at work on the leavage previously."

In 1904 Macdougall(2) records damage in Aberdeenshire and states "It is interesting that, as far as I know, all the complaints that have been made of the destructive work of this beetle (*H. rugosus*) and its grub have reference to Aberdeenshire."

He says "The leaves may be eaten, the leaf stalks may be holed and tunnelled; the swollen root tubers may be irregularly gnawed and tunnelled on the outer surface, especially at the upper part. The favourite place for the pests is at the crown of the tuber, sheltered among the leaf bases, the young leaves being destroyed."

In November, 1911 and 1912, Macdougall(3) and (4) reports that he found the grubs feeding in the heart of the youngest central leaves, and adds "It is not often that this insect proves troublesome."

The English records are as follows:

In 1906 it was reported to the Board of Agriculture as having done considerable damage to turnips on a farm in north Lincolnshire, and the attacked turnips were described as "stunted in growth, hard and woody, and full of galleries."

Again in November 1912 the larvae were found damaging several large patches of turnips at Chichester.

On December 21st, 1921, Mr W. P. Bocock of Gazeley, Newmarket, Suffolk, sent me some turnips badly damaged by *Helophorus* larvae, with the following observations: "After harvest the crop looked well and flourishing, now there are none left at all on 10 acres."

On December 29th I visited this field, a light boulder clay on a gravelly subsoil, containing 30 acres of turnips, 10 acres of which were completely ruined by the combined attacks of the larvae of *Helophorus rugosus* (and possibly *H. porculus*) and *Psylliodes chrysocephala* and subsequent rotting. The bulk of the damage was due to the feeding of *Helophorus* larvae. In the remaining 20 acres many large patches on the lower side of the field were also spoiled. *The turnips were sown about the middle of July.* In the same field, next the turnips, swedes were attacked by *Helophorus* larvae, but not nearly so badly as the white turnips. Galls due to *Ceuthorhynchus pleurostigma* were however very numerous, much more so than on the turnips. Beyond the swedes a

piece of thousand-headed kale was not damaged at all by the *Helophorus* larvae.

On December 31st, 1921, I examined the turnips exposed for sale in Cambridge and found that a large percentage were damaged in the same way. I then examined fields near Cambridge and found *Helophorus* larvae and their damage in a large percentage of the turnips.

On the University Farm on a gravel soil a piece of white turnips sown on August 8th, 1921, after oats was badly attacked by this pest together with larvae of *Psylliodes chrysocephala*, the latter being rather more numerous. The turnips were left unthinned, about 90 per cent. were attacked by *Helophorus* with the result that about 40 per cent. rotted. Alternating with these turnips were strips of thousand-headed kale which were not attacked.

On two neighbouring fields of white turnips, sown on August 18th and September 12th respectively, the attack was very slight, but larvae of *Psylliodes chrysocephala* were fairly abundant.

On July 1st, 1922, a small strip of white turnips was sown on the University Farm on the land where the turnips had been badly attacked the previous year, but these did not suffer nearly as much as expected from attacks of *Helophorus*.

In 1922 at my suggestion Mr Bocock did not plant turnips in fields adjoining the 1921 crop. In December he wrote, saying, "The grubs have done no damage to my turnips this year."

At Drainage Farm, Bourne Fen, Lincolnshire, in January 1923, 7 acres out of a 17-acre plot of turnips (grown for seed) were spoiled by *Helophorus* larvae and were ploughed up. This field was of interest in showing the effect of the cropping on the damage caused by this pest. The 7-acre portion was cropped in 1922 as follows: early potatoes, which were a failure, were followed by a mixture of turnips, coleseed and mustard ploughed in as green manure. The turnip-seed crop was sown on September 28th, 1922.

The 10-acre portion which was free from attack was cropped in 1922 with Majestic potatoes which were a good crop.

During the winter of 1923–4 I examined a number of fields and allotments containing turnips, and in nearly all of them *Helophorus* larvae were present and in many cases the turnips were badly nibbled but not bored into nearly so much as in the case of the attack in 1921.

On the University Farm stubble turnips (after peas) drilled on August 3rd, 1923 (in the field where the turnips suffered badly early in 1922),

were attacked to the extent of 100 per cent. Rape adjoining these turnips drilled on July 12th, 1923, was not nearly so badly attacked.

In the next field rape drilled on July 26th, 1923, was slightly attacked but no damage was found on the adjoining thousand-headed kale drilled the same day.

On February 7th, 1924, I visited seed beds containing swede plants for seed growing in south Lincolnshire and in several cases found *Helophorus* larvae and their nibblings at about the ground level.

Nearly all the turnips offered for sale in the shops in Cambridge during March 1924 were marked by nibblings of this pest.

On September 25th, 1924, I visited a farm belonging to Mr W. J. Serjeant, Werrington, near Peterborough.

In Field *A* swedes were sown up the middle of the field on June 16th and on July 25th to 27th. White turnips were sown on each side of the swedes. About 10 to 12 acres of the turnips were almost a complete failure through attacks of *Helophorus* larvae, whereas the swedes were only slightly attacked. On this field the swedes were badly attacked by "Finger and Toe," *Plasmodiophora brassicae*, whereas the turnips were *entirely* free from this disease although the nearest turnips were only 2 ft. from the diseased swedes. The previous rotation was 1920 turnips, 1921 barley, 1922 oats, 1923 wheat.

In Field *B* white turnips sown July 25th to 27th. Three acres out of 6 were spoiled.

In Field *C* were 4½ acres of turnips sown July 25th to 27th, and 2 acres were spoiled, whereas the 4½ acres of swedes although slightly attacked were almost a normal crop.

Mr Serjeant first noticed the trouble in the turnips early in September. The soil is a light loam on a gravel subsoil.

On a neighbouring field after allotments white turnips drilled July 24th to 27th were attacked in patches.

In the early stages of this work the beetles I examined were all *Helophorus rugosus*, and I assumed that only one species was present on the turnips. In 1924, however, I noticed a number of *H. porculus* among the specimens which I had reared, and in March and April of 1926 I collected a number of larvae from turnip and rape from which I bred out thirty-seven adults of *H. rugosus* and forty-two adults of *H. porculus*.

I made a careful examination of a number of these larvae in order to find some means of separating them but without success. I also compared these larvae with those of *H. nubilus*[1], and except that the average size of the latter is smaller I could find no constant difference.

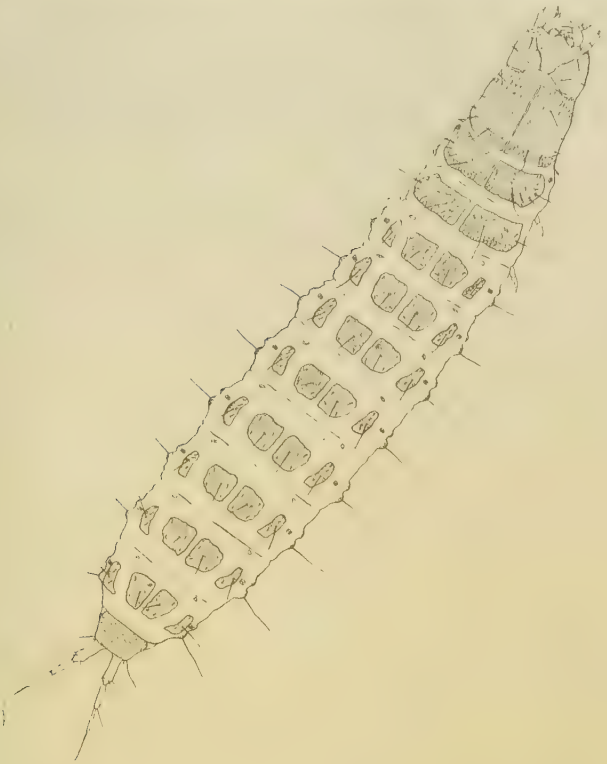
[1] I have found the larvae of this beetle injuring wheat in Cambridgeshire and Lincolnshire.

Helophorus rugosus.

Adult (Plate XXXVIII, fig. 1). Length 4½–5½ mm.

The external characters are usually masked in collected specimens by a covering of mud. Sharp's(5) explanation of this is as follows:

"I must emphasise the fact that on studying *Helophorini* it is necessary to have well cleaned specimens. These insects secrete on the surface of



Text-fig. 1. Larva of *Helophorus rugosus* (*Helophorus porculus* is similar).

the body a peculiar exudation which dries, and obscures the smaller points of structure even in cases where the specimen has the superficial appearance of being quite clean; while in other cases it retains foreign bodies, so that the specimen is covered with a sort of incrustation.

Specimens are best cleaned by soaking in very hot water, then washing them with soap, and afterwards with benzine."

Head, dark reddish-brown, covered with short white hairs, retractile into thorax.

Clypeus, rounded in front and not margined (this is a distinguishing character from *H. porculus*).

Antenna, pubescent—9-jointed, the four terminal segments forming a club.

Pronotum (Plate XXXVIII, fig. 2) large, with median portion reddish-brown, strongly arched; sides yellowish and form flanges. The raised median portion forms a sort of hood over the head. Its front margin is very irregular in outline, being convex in the middle, then concave on each side, then sloping forward again to form prominent anterior angles.

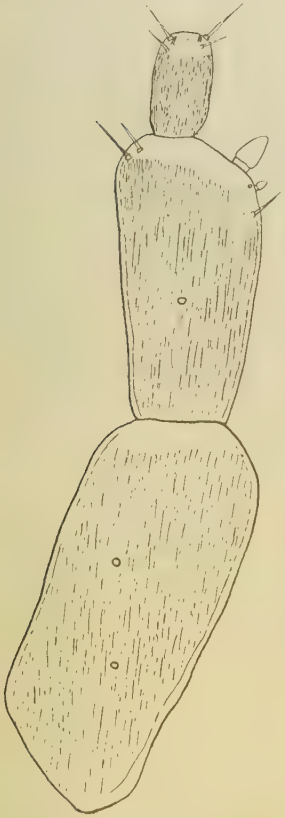
The raised median portion of the pronotum is marked by three grooves with pronounced warty prominences between them. The median groove is straight but of varying widths. The submedian groove is irregular and divides the interval exterior to it into two prominences. The two pairs of grooves and the pair of intervals outside the raised arch are not prominent.

Elytra, brownish, testaceous, with dark markings as shown in Plate XXXVIII, fig. 1, slightly broader than thorax, humeral angle projecting. There are ten punctured striae visible from above on each elytron, and in addition a short stria of a varying number of punctures with its accompanying ridge at the base between the first and second striae. Alternatively this short stria can be regarded (as Sharp has done) as being between the first stria and the suture, but there is a short ridge outside the short stria and connected with the suture. Outside the tenth stria is a marked projecting ledge which at first looks like the edge of the elytron. On turning over the elytron the eleventh stria can be seen on the shining space between the ledge and the edge of the elytron. The elytron is markedly ridged in alternate interstices, the middle ridge being formed by the inner borders of the elytra. Each ridge bears a row of short brownish hairs and the alternate interstices are smooth. None of the ridges reaches the outer border of the elytron which forms a flange almost at right angles to the body and is fringed with short hairs.

Eggs. Although a number of beetles were kept in pots for the purpose of obtaining eggs, copulation was never observed and no eggs were found.

Larva[1] (Text-fig. 1).

The larva, when fully grown, measures about 1 cm. in length and nearly 2 mm. in width. It is cylindrical, tapering gradually towards the head end and more sharply at the tail end, its widest part being the



Text-fig. 2. *Helophorus rugosus.* Right antenna of larva.

Text-fig. 3. *Helophorus rugosus.* Left mandible of larva.

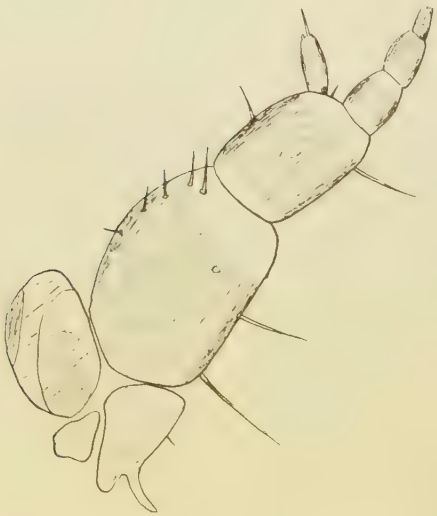
sixth to the seventh abdominal segments. It is soft and fleshy, cream coloured and with very characteristic brownish chitinous plates on the thoracic and abdominal segments.

The *head* is narrower than the prothorax.

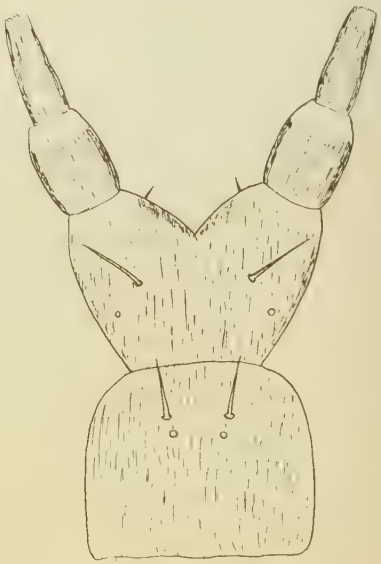
[1] This description also applies to *H. porculus* except as regards size.

The *antennae* (Text-fig. 2) are of moderate length, composed of three segments of a yellowish-brown colour. The basal segment is the longest. The second segment is widest near the distal end and then narrows abruptly to the apex, near which are three sensory papillae of varying sizes and three setae. The terminal segment, which is small, bears six setae of varying sizes at its apex.

The *mandibles* (Text-fig. 3) are stout and brown in colour; each bears three bluntly pointed teeth, the larger terminal one bearing a varying number of serrations on its inner edge. These serrations are sometimes



Text-fig. 4. *Helophorus rugosus.* Right maxilla of larva (from above).



Text-fig. 5. *Helophorus rugosus.* Labium (from below) of larva.

worn down. The other two teeth are hooked, and just below the lower tooth is a pseudomolar process bearing two or three teeth. Below this process and near the inner basal angle are a small bunch of fine setae. On the outer edge a well-developed spine is present.

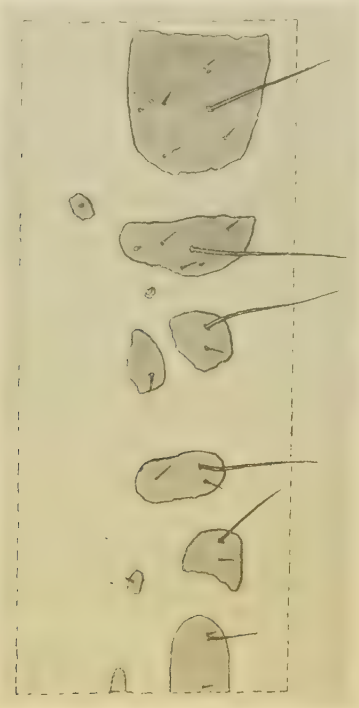
The *maxilla* (Text-fig. 4) consists of a cardo divided into three sclerites, a stipes and palpiger of which the former is the larger. A small lobe is present near the apex of the palpiger on its internal side and a rather short triarticulate maxillary palp on its external side.

The labium (Text-fig. 5) consists of an oblong mentum, rather broader

than long, with the anterior corners rounded, a prementum with a cleft, no ligula and two fairly well developed labial palps.

The *labrum* appears to be represented by a conical process fused with the frons and clypeus and bearing small papillae on its sides.

Just behind each antenna is a group of six *ocelli*.



a *b* *c*

Text-fig. 6. Plates and spiracle on the left side of abdominal segment 5 of *Helophorus rugosus* (*a*, *b* and *c* are halves of median ventral plates).

Thorax. The prothorax is trapezoidal, slightly narrower in front and twice as long as the meso- and metathorax. The scuta of the three thoracic segments are thickly chitinised and brown in colour and apparently divided down the middle line into two large plates on each segment.

The legs are short and terminate in a simple claw.

Abdomen. The abdominal segments are nine in number, the first eight being very similar in size and markings. The chitinous plates on segment 5 are shown in Text-fig. 6. These plates with their long setae are a characteristic feature of *Helophorus* larvae. In segment 1, plate (*a*) is much larger and plate (*b*) is joined to the small plate above it. The dorsal

Text-fig. 7. Pupa of *Helophorus rugosus*.

and lateral plates are of a smoky brown colour and the ventral plates are much paler. The dorsal surface of the ninth segment is completely covered by the anal plate. The ninth segment also has a pair of lateral plates with one long, two medium and one short setae, and a ventral plate with two pairs of long and one pair of short setae.

The anal cerci (Text-fig. 1) are long and three jointed. The basal

segment is the widest and bears near its apex three long setae, one on the dorsal and two on its ventral surface. The middle segment bears near its apex one long ventral seta, but I have one specimen in which the middle segment of the right cerci bears two long ventral setae. The terminal segment which is the narrowest bears one long seta at its apex and just behind it one very small seta.

Pupa (Text-fig. 7).

The pupa is soft and almost white in colour. It measures about 6 mm. in length. It bears a number of long bristles, all of which arise from prominent conical tubercles.

The head is bent beneath the prothorax and bears three pairs of long bristles.

The prothorax is large and somewhat similar in shape to that of the adult. Its surface is somewhat sulcate and there is a longitudinal groove in the mid dorsal line. It bears eight pairs of long bristles.

The mesonotum has a semicircular protuberance in the middle line, near its posterior border, on each side of which is a long bristle.

The metanotum carries one pair of long bristles.

There are ten abdominal segments, but the tenth, which consists of two lobes, is only visible from the ventral surface.

Segment 1 has one pair of dorsal and one pair of lateral (epipleural) bristles. Segments 2 to 7 each have one pair of dorsal, one pair of epipleural and one pair of pleural bristles.

The number of bristles on segments 8 and 9 seems to vary. Usually segment 8 has one pair of dorsal and one pair of pleural bristles, whilst segment 9 has one pair of dorsal bristles. I have one specimen in which there is in addition a pair of epipleural bristles on segment 8 and a pair of pleural bristles on segment 9.

The ninth segment terminates in a pair of pointed lobes.

Distribution.

Sharp (5) says "It occurs not only in Britain but appears to be widely distributed in the Mediterranean region. I have specimens from France (Alsace, Pyrenees, etc.), Spain (Albarracin), Algeria (Biskra, etc.), Tangier, Malta, Thasos Island, and Besika Bay. In our own country it has been recorded from England, Scotland, Ireland and Wales, and also has been described as injurious to turnips; but most of these records probably refer to *H. porculus*. Reitter does not distinguish the two as occurring in Germany, and mentions only *rugosus* (in Westdeutschland, sehr selten)."

Fowler says "Rather local, but widely distributed throughout England and Wales, both inland and near the coast; not so common towards the north; Scotland, scarce, Lowlands, Tweed, Forth, Solway, and Dee districts."

Macdougall(2) says "It is found in several widely separated districts in Scotland," and he also records cases of damage from Aberdeenshire but from no other district in Great Britain.

Miss Ormerod's records of damage are all from Aberdeenshire.

In the winter of 1921–2 I found large numbers of the larvae feeding on turnip bulbs in West Suffolk and around Cambridge. In the winter of 1922–3 they were much less plentiful in these districts, but large numbers were found near Spalding (Lincs.) feeding on young turnip plants. Since then I have found the larvae of this or *H. porculus* in Lincolnshire, Norfolk, Cambridgeshire, Suffolk, Essex, Bedfordshire and the Soke of Peterborough.

Fox-Wilson records it as attacking lettuces in Surrey, and a single specimen attacking a wallflower in Hampshire.

Roebuck has found it in Shropshire and Staffordshire, and Hodson has found adults in turnips in Devon.

Theobald says it occurs in very scanty numbers in the south-east.

Helophorus porculus.

Adult (Plate XXXVIII, fig. 3). Length 3·8–4·7 mm.

Sharp says "This species has been, and still is, confounded in collection with *H. rugosus*, though the two are not really closely allied, *H. porculus* being distinguished from *rugosus* and from all other *Helophorini* by the shape of the front of the head. (*The clypeus is subtruncate and raised and margined behind the labrum.*) In addition to this and that the submedian interval of the pronotum is not completely divided into two parts, there exists also a distinction in the suprapleural area which is narrower in *porculus*. It appears to be a variable species."

Fowler says "From *H. rugosus* Ol. (for which we ought perhaps to substitute the name *H. rufipes* Bosc.) the species may be known by its average smaller size, flattened dorsal costae of thorax, and the fact that the elytra are not sinuate near the base, *and have the humeral angles rounded*, whereas in *H. rugosus* the elytra are sinuate *before* the base *and the humeral angle is turned outwards forming a distinct tooth.*"

Larva.

I made a careful examination of a number of mixed larvae of *H. rugosus* and *H. porculus* but I was unable to find any characteristic difference between them, so that my description of the larva of *H. rugosus* applies to this species except as regards size.

Distribution.

It extends from the north of Scotland to the Mediterranean (Sharp).

According to Fowler it has been found in Surrey, near Oxford, in Norfolk, Isle of Wight, at Bradfield, the Scilly Isles, and Garve, N.B.

I have only definite records for Cambridgeshire where I have found it in company with *H. rugosus* in allotments and fields near Cambridge.

Hosts.

These pests feed chiefly on plants belonging to the genus *Brassica*, but are not confined entirely to this genus.

The larvae of both species show a decided preference for the common white turnip (*Brassica rapa*), and wherever these are present they are much more severely attacked than other plants.

Swedes (*B. campestris rutabaga*) and rape (*B. napus*) also suffer from moderate attacks.

Cabbages (*B. oleracea capitata*) are not often attacked, but in one case I found larvae boring into the galls made by *Ceuthorhyncus pleurostigma* on the roots.

Thousand-headed kale (*B. oleracea* var. *acephala*) is not usually attacked, but I have seen slight injury from larval attacks near the ground level.

In December, 1924, Fox-Wilson sent me a specimen of a larva of *Helophorus* sp. with the following note:

"The enclosed *Helophorus* larva was found mining the stem of wallflower attacked by *Plasmodiophora brassicae* sent from Portsmouth; all the six plants submitted were mined in a similar manner to the enclosed portion of stem, and three larvae were found in the plants."

In June 1926 I found both species under the basal leaves of shepherd's purse (*Capsella bursa-pastoris*) in a field of sugar beet following a crop of turnips and rape which had been attacked by the larvae. On March 10th, 1924, I found a single specimen of an *Helophorus* larva (which on rearing proved to be *H. rugosus*) boring holes in a winter bean plant in a field of beans on heavy land, bordering fenland in Cambridgeshire. This damage was very similar to wireworm damage and was repeated

in the laboratory when the larva was placed on a young seedling bean plant.

On February 6th, 1923, a single larva was found apparently feeding on the bud of a sainfoin plant near Cambridge.

The adults also feed on the leaves of white turnips and probably other plants.

Mr G. Fox-Wilson of Wisley has kindly sent me particulars of an attack in 1924 on lettuce by *Helophorus* sp. at Walton, Surrey.

The soil was heavily manured in 1922 and cropped with sea-kale, which was lifted in November for forcing.

One ton of lime per acre was then applied and turnips sown on March 16th, 1923. These were marketed in June.

After the turnips a fair crop of French beans was grown.

The field was then heavily manured, ploughed and fallowed during the winter.

In the spring of 1924 one ton of carbonate of lime was applied. The soil was rolled light, and Cos lettuces were planted the last week in January. (These had been sown in cold frames the previous October.)

As soon as the lettuces had made fresh root, the *Helophorus* larvae bored into the bases of the stems and burrowed upwards, emerging at the hearts, whereupon the lettuces collapsed.

The grower adds "I have noticed this same beetle in all my Cos lettuces planted this year—it is certainly worse in the fields where turnips and sea-kale have been grown previously. I have had more trouble this year with soil bugs than I have ever had before during my 20 years' experience. The soil is certainly in a very high state of cultivation."

Notes on the life histories.

On February 15th, 1922, twenty-four larvae were collected and put into three pots of soil containing transplanted turnips and kept in the laboratory. From time to time other larvae, and later on pupae, were collected and also put into pots of soil in the laboratory. At the same time observations were made on the various stages present in the field.

In the laboratory pupation began on March 21st and three adults were found on April 24th. The last pupae completed the change on May 31st.

An examination of the turnips on the University Farm sown on August 8th, 1921, showed that the larvae continued to attack the turnips up to the end of January. After that date very little damage

was done, but the larvae remained in the soil until May 18th. (The larvae make small chambers in which to pupate.) The first pupae were found on May 10th and I continued to find them until the end of May.

I had great difficulty in finding beetles owing to their covering of soil and to their habit of remaining quiescent. Even when examining soil from pots in which I had placed the adults it was no easy matter to find all the beetles present, so that the great difficulty of finding them in the field can easily be imagined.

During searches in June and July only occasional beetles were found in the soil although previously I had no difficulty in finding a number of pupae in the same plot in May.

The beetles were kept in the laboratory in pots in which young turnip plants were growing. They were rather sluggish and spent most of the time in the soil. Occasionally one came above ground, climbed up a turnip plant and ate portions of the young leaves or made holes in one of the older leaves. Most of the beetles lived in the pots until October, and three of them lived until December.

Copulation was not observed and no eggs were found.

On October 6th, 1922, five larvae about 6 mm. long and two about 9 mm. long were found feeding on the turnips sown on July 1st on the edge of the plot of turnips previously attacked.

The larvae continued to feed on the turnip bulbs right through the winter until the middle of February, and on March 20th, 1923, three were found feeding at the base of the leaf stalks and in the neck of the plant.

When sowing oats (after turnips) on March 15th I found larvae feeding on turnips lying on top of the ground. Two adults were also found in March, but as no pupae had been found up to this date these must have lived through the winter. Eggs are probably laid in July and August, as attacks of the larvae on turnips have been noticed early in September. On September 25th, 1924, larvae were not full grown, measuring only 3 to 7 mm.

The larvae continue to feed throughout the winter until the end of March or even later, but some of them seem to leave the plants earlier than this. The latest date on which I have found them is May 10th, 1926. In the soil they hollow out a small chamber about 2 in. below the surface in which to pupate. Pupation takes place in April and May and in this stage they exist for about three weeks. In 1926 near Cambridge the first pupa was found on April 8th, but in 1925 no pupae were found in April, the first being found on May 6th.

In 1924 the first pupa was found on April 29th. The first beetles were found on May 28th in 1924, June 5th in 1925, May 26th in 1926. The beetles continue to live throughout the winter and may be found in any month up to March. The latest date I have found them is March 20th, 1923. They are very sluggish and appear to feed chiefly on leaves. They do not appear to eat much, judging by those which were kept in the laboratory until December.

Time and nature of injury.

Both the larval and adult stages cause injury to plants.

In the larval stages the damage is done during the late summer, autumn and winter.

The most obvious damage to turnips (Plate XXXVIII, fig. 5) consists of irregular gnawings and channelings on all parts of the "bulb," but in the case of a slight attack chiefly at the ground level. In addition deep holes are often made and larvae may be found partly or entirely in the burrows. This damage to the "bulb" may allow the entrance of other organisms which set up rotting. This rotting was much more noticeable in the spring of 1922 than in 1924–6. At the same time as damage is being done to the "bulbs" other larvae may be found feeding in the crowns of the plants, and eating away the young developing leaves or making large tunnels in the leaf bases. This latter damage should not be confused with the damage caused by the flea beetle *Psylliodes chrysocephala*. Serious damage necessitating the ploughing up of the crop may be caused in the early part of the year to turnips planted out for seed growing. This damage is caused by the larvae eating away the young developing leaves and growing point and so ruining the plant for seed purposes (Text-fig. 8).

Young swede plants for seed growing may be injured whilst still in the seed bed by the nibblings of the larvae. Small holes are often made which may serve as a source of infection of *Pseudomonas campestris* and *Phoma lingam* which are associated with the so-called "swede canker" in which the flowering stalk rots at the base and breaks off or falls down. The larvae of *Psylliodes chrysocephala* are probably worse offenders than *Helophorus* larvae in causing this trouble.

Occasionally in the case of both turnips and swedes larvae may be found feeding inside the stem at the base of the young flowering stalk.

The ordinary rotation swede crop suffers similar damage to that of the turnip described above, but the attack is usually much less severe, which may in part be due to the earlier sowing of this crop as compared

with turnips. A moderately bad attack is possible on rape, and in this case the main roots and hypocotyl are gnawed (Plate XXXVIII, fig. 4) and the crown of the plant eaten.

Text-fig. 8. Young white turnip plant (for seed) damaged by *Helophorus* larvae.

With kale only slight gnawings just below ground level have been met with, and cases where this crop has been free from attack have been noticed in fields where the turnips were badly injured.

In the case of cabbages the damage consists chiefly of boring into galls made by *Ceuthorhynchus pleurostigma*. Isaac(6) says "The larva (of *H. rugosus*) has often been seen to bore right into the gall (caused by *C. pleurostigma*) casting away the plant tissues bitten out in the process, and thus making a clear effort to get at the weevil larva inside.

More than once they have been taken with the weevil grubs between their mandibles, and when kept in cages with galls they burrow and empty the galls of the occupant and also feed on the inner tissues."

I repeated this experiment *without success*; *and field observations* provide very little evidence that the larvae are of much value in reducing gall weevil.

Only one case of damage to beans has come to my notice, and in this case the damage was somewhat similar to wire-worm damage, the larvae making holes in the young stem just above the ground level.

Lettuces may be killed by the boring of the larvae into the base of the stem and then burrowing upwards and emerging in the centre of the heart.

In the *adult stage* injury is caused during the summer, chiefly when the turnips are young. I have only found damage to turnip plants and this not of a serious nature, although reports from Scotland show that it is more severe there.

The beetles feed in the crowns of the turnips and eat the developing leaves or make holes in the older leaves.

Control.

From the above observations it would appear that a severe attack is most probable with very late sown turnips. No case of a bad attack has been noticed with turnips sown before July.

The following are the dates of sowing in the cases where the damage was severe:

District	Crop	Date of sowing
Gazeley, near Newmarket	Rotation turnips	Middle of July 1921
Werrington, near Peterborough	Rotation turnips	July 27th–29th, 1924
Cambridge	Turnips as catch crop	August 8th, 1921 August 3rd, 1923 August 26th, 1925
Holbeach Marsh, Lincolnshire	Turnips for seed	September 28th, 1922

Turnips sown at the normal time of sowing do not appear to be seriously affected, so, in districts where this pest has proved troublesome, an effort should be made to get turnips sown in good time.

The above observations also indicate that the liability to attack is increased when cruciferous crops are grown at frequent intervals.

Turnips should not be grown on land adjoining that in which a piece of turnips was attacked the previous year, and in no case should a crop liable to attack follow one which had been attacked the previous year. In Lincolnshire only the plants following other cruciferous crops suffered damage to any extent, those following potatoes in the same field being comparatively free from attack.

Cabbages and kale do not appear to suffer much damage from these pests, probably due to the nature of their stems. This probably applies also to cauliflowers, broccoli and kohlrabi.

Lettuces should not be grown on infected land.

The seed beds of turnips and swedes raised for seed growing should be on land which has not grown a cruciferous crop for at least one year as insect injury (including these pests) in the seed beds appears to be the primary cause of so-called "swede canker."

Turnips and swedes for seed growing should not be planted out on land following a cruciferous crop unless this crop has been examined and found to be free from attack.

As the larvae are feeding and growing in late summer a late summer fallow should suffice to starve any larvae present. Cultural operations are likely to have the greatest effect if carried out when these pests are mostly in the pupal stage, *i.e.* about the latter half of May in the east of England.

Most of the pupae are in the top $2\frac{1}{2}$ in. of the soil.

I am greatly indebted to Mr A. W. Rymer Roberts for his assistance in describing the mouth-parts of the larva.

Summary.

Helophorus rugosus Ol. and *Helophorus porculus* Bedel. have caused serious damage to late sown white turnips in several cases in the east of England during the last few years.

A case in which the growing points of white turnips (planted out for seed growing) were eaten out by the larvae is recorded.

Swedes and rape are damaged by these pests but not to the same extent as white turnips.

Kale and cabbages are only slightly damaged.

Lettuces were seriously damaged in Surrey in 1924.

One case of an attack on beans by *H. rugosus* was also noticed. Most

of the damage is caused by the larvae which gnaw or tunnel into the "bulb" of the turnip as well as feeding on the developing leaves. The adults also feed on the leaves.

Eggs are probably laid in August and the larvae live from August until April or May.

Pupation takes place in April or May and pupae live about three weeks.

Adults were found from May until the following March.

Descriptions of the larval, pupal and adult stages are given.

Late sowing and frequent cruciferous crops appear to predispose plants to attacks.

REFERENCES.

(1) Ormerod, E. A. Reports for 1889, 1894 and 1895.
(2) Macdougall, R. S. (1905). *Trans. High. and Agr. Soc. of Scot.* p. 221.
(3) —— (1913). *J.B.A.* p. 41.
(4) —— (1913). *Trans. High. and Agr. Soc. of Scot.* p. 195.
(5) Sharp, D. (1915, 1916). Studies in Helophorini. *Ent. Mo. Mag.* monthly articles.
(6) Isaac, P. V. (1923). *Ann. App. Biol.* x, no. 2, p. 168.

EXPLANATION OF PLATE XXXVIII

Fig. 1. *Helophorus rugosus.* ×4.
Fig. 2. Pronotum of *Helophorus rugosus.* ×9·4.
Fig. 3. *Helophorus porculus.* ×4.
Fig. 4. Damage to rape by *Helophorus* larvae.
Fig. 5. White turnip severely damaged by *Helophorus* larvae.

(*Received January 24th*, 1928.)

Fig. 1.

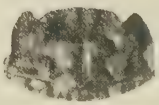


Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

PETHERBRIDGE.—The Turnip Mud Beetles (pp. 659–678).

THE METAMORPHOSIS AND BIOLOGY OF *RHYNCHAENUS ALNI* L. (COLEOPTERA)

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(With 22 Text-figures.)

Classification.

Order: **COLEOPTERA.**
Superfamily: RHYNCHOPHORA.
Family: Curculionidae.
Genus: *Rhynchaenus* Clairville, 1798.
(*Orchestes* Illiger, 1804.)
(*Salius* Schrank, 1798.)

Introduction and historical review.

The material on which this paper is based was collected in the summer of 1925 and spring of 1926 in the neighbourhood of Warwick, England. The larvae were very common and found in abundance on the elms of the district where the trees were suffering from the damage done by the insect, hardly a single tree having escaped their ravages.

While the biology and metamorphosis of some of the species of the genus *Rhynchaenus* (syn. *Orchestes*) is known, of others our knowledge in this connection is somewhat scanty. Trägårdh (1910) contributed much valuable information towards our knowledge of *Rh. populi* Fabr., *Rh. fagi* L. and *Rh. quercus* L., and this paper is intended as an additional contribution to our knowledge of *Rh. alni* L.

The species under consideration is not only a miner, in its larval state, on *Alnus* as the specific name of the insect leads one to conclude but also is an enemy of *Ulmus* and, as far as can be ascertained from the literature and from observations, it appears to occur more commonly as a pest of elms. It is not surprising, therefore, that in the literature dealing with this weevil there has been much misunderstanding and a certain degree of controversy over the host plant and hence over the specific name.

The first description of the insect was made in 1758 by Linnaeus. In a later edition of *Systema Naturae*, in 1767, a further note was made by the same author on this beetle, the host plant in both instances being *Alnus*.

De Geer, in 1775, described two weevils one of which he called "Curculio saltator ulmi." The adult was described and a note on the habit of life of the larva, which was found mining in elm leaves, was added, while the pupation was also mentioned. The second weevil, named "Curculio saltator alni," was briefly described from alder. In 1795 Herbst mentioned that this beetle mined in the leaves of alder, while Bertoloni, in 1844, discoursed at length on the weevil. This latter author reared the adults from larvae which were found infesting the leaves of *Ulmus campestris* and described in detail the damage caused; the larva, pupa and adult also are described briefly and the account is concluded by an interesting note in which the writer was particularly desirous of discovering some methods of fighting the pest which was ravaging so widely the foliage, extensively used for fodder. Nineteen years later, in 1863, von Frauenfeld contributed a note on a weevil which he called *Orchestes ulmi* De G. This insect he had shown to Redtenbacher who called his attention to De Geer's description of "Curculio saltator ulmi"; while Miller, who also was shown the specimen, was inclined to assign it to *Orchestes alni*. Von Frauenfeld, however, gave a detailed description of the adult and a note on the larva and pupa and, while he was not inclined to agree with Miller's identification, it seems probable he was dealing with one of the varieties of *Rh. alni*. In fact, in 1874, Kaltenbach noted that the insect under consideration had been reared from both elm and alder leaves and suggested that von Frauenfeld, who had described the life history of *O. ulmi* De G., had had in reality *O. alni* before him. Several writers have, within the past fifty years, added notes on the biology and descriptions of the insect, and it is now well established that *Rh. alni* L. is a leaf-miner of both elm and alder, at times producing a great deal of damage to the foliage of the former tree, or to both.

According to Frost (1925) there are 58 species of *Orchestes* described, occurring in North America, Europe and Siberia, of which 21 are leaf-mining species whose habits are well known. *Rh. alni* L. has a fairly widely spread distribution and is known to occur in many parts of Europe. In Britain it occurs very abundantly from the Midland districts southwards, especially in the south of England and South Wales, but is much rarer further north. It attacks both *Ulmus campestris* and *Alnus glutinosa*.

THE ADULT.

The weevil (Figs. 1 and 2) is of insignificant size and appearance and, while it is well known to systematists, the writer has deemed it advisable to include in this paper a description for those who are not so well acquainted with this insect.

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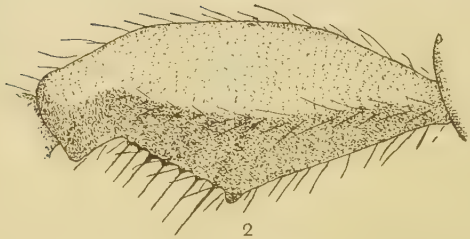

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Fig. 1. *Rhynchaenus alni* L., adult, dorsal aspect. ×22.

Fig. 2. Inner surface of posterior femur of adult to show angulation of surface, central tooth and row of rigid cilia. ×55.

Fowler's description (1891) of the adult is as follows:

"*O. alni* L.—Black, pubescent, with the antennae, tarsi, thorax, and segment of the abdomen and elytra rufo-testaceous, the latter with four black spots, which are very variable, the apical ones often being united at suture, often very obscure, and sometimes wanting; antennae with scape long, inserted just behind middle of rostrum; thorax closely and rather strongly punctured, sometimes with an abbreviated black patch on the middle of disc; elytra with strong punctured striae; posterior femora very strongly thickened, with a tooth in the middle and a series of rigid cilia behind it. Length $2\frac{1}{2}$–3 mm."

The egg.

The egg is oval in shape, 0·65 mm. long and 0·3 mm. broad at the middle, with the ends somewhat abruptly rounded. In colour it is whitish or creamy with a faint trace of a greenish tinge. The eggs are deposited singly by the female beetle, as a rule within the mid-vein or one of the larger secondary veins, on the underside of the leaf in a cavity which is bitten out by the adult. Thus the egg is not visible unless it is very carefully dissected out from the leaf-vein tissue.

The larva.

1. *The first stage larva.*

The first stage larva (Fig. 3) attains a length of 0·7 mm. and a width at the prothorax, which is the broadest part of the animal, of 0·3 mm. The colour is yellowish-white and the body is devoid of any markings, while the cuticle is quite smooth. The intersegmental constrictions are shallow and there are no lateral projections. The form of this larva differs considerably from that of the later stages, since the body is widest at the anterior region, across the prothorax, and gradually tapers towards the posterior extremity; it is of fairly even width, however, throughout about half its length. In this stage the tergite of the prothorax is not developed as a plate and the head is not enveloped by the anterior edge of the segment, as in later stages, but remains quite free. There are no ventral thoracic plates or markings.

2. *Intermediate larval stages.*

After the first moult the larva assumes generally the characters, which are detailed below, of the full-grown larval stage. The tergite of the prothorax becomes developed as a plate enveloping the posterior

region of the head capsule, while the thoracic sternites acquire the chitinous plates and circular patches described under the full-grown larva. The thoracic region increases in width, and the head becomes larger, and the animal assumes a more tapering form while the intersegmental constrictions become more pronounced. During its further growth the larva shows no further marked changes and gradually acquires the form of the full-grown larva, a description of which follows.

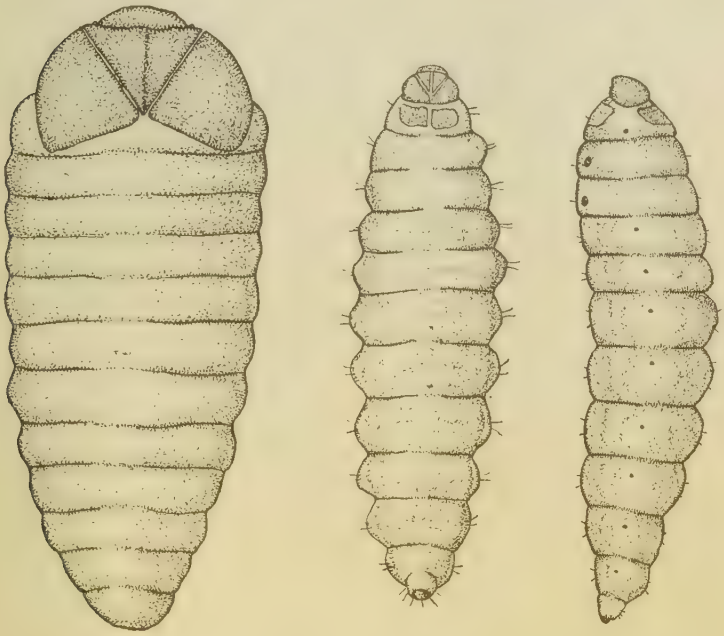


Fig. 3. First stage larva, dorsal aspect. ×135.
Fig. 4. Full-grown larva, dorsal aspect. ×18.
Fig. 5. Full-grown larva, lateral aspect. ×18.

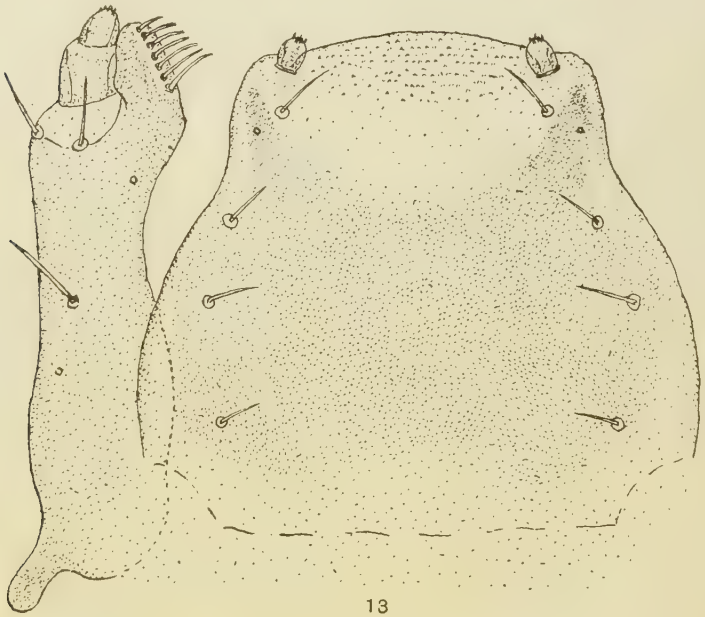
3. *The full-grown larva.*

The full-grown larva (Figs. 4 and 5) attains a length of 4·5 mm. It is cream-white, the head capsule and parts of the thoracic segments being brown in colour.

The body is more or less cylindrical with a slight taper towards head and hind ends. The larva is widest across the middle of body in the

third abdominal segment and here measures 1·3 mm. The spiracles, which are small and dark brown, occur on the prothorax and abdominal segments 1–8, and are situated on the mid-lateral line. There are no legs present.

The head. The head (Figs. 6 and 7) is of a dark chestnut-brown



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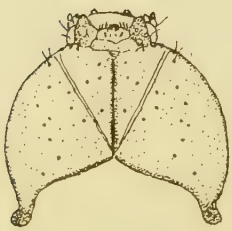

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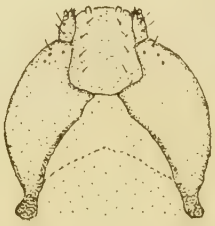

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Fig. 6. Head capsule of full-grown larva, dorsal view. ×55.
Fig. 7. Head capsule of full-grown larva, ventral view. ×55.
Fig. 13. Full-grown larva: right maxilla and labium, ventral aspect. ×360.

colour and its base is partly enclosed within the prothorax. The head capsule, which is curved slightly downwards, is flattened dorso-ventrally. The visible part is almost semicircular in outline. The posterior margin is deeply excavated, the posterior angles projecting strongly within the prothorax.

The frons is largely developed and occupies about one-third of the upper surface of the capsule projecting backwards almost to the hind margin. In shape, it is triangular, as broad as long, the anterior edge being slightly thickened. The two halves of the vertex are separated from it by a pair of narrow lines of almost transparent cuticle. Interiorly, a dark median longitudinal ridge is seen on the frons.

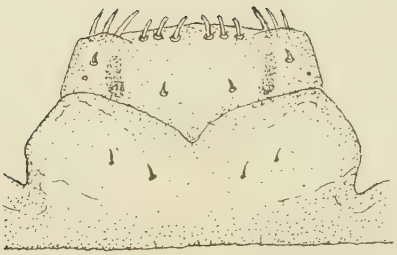
The sclerites of the vertex project backwards to form a convex hind margin to the capsule.

The clypeus (Fig. 8) is very broad and short; the sides are straight and converge forwards and a median incision divides it into two lobes. The anterior part, overlapping the labrum, is thin, but the sides are well chitinised. The anterior lateral part of each lobe is somewhat thickened. Two small hairs occur laterally about the middle.

The labrum (Figs. 8 and 9) is about twice as broad as it is long, the anterior edge being slightly convex. The straight lateral edges converge forwards. The posterior margin has a median triangular projection. Dorsally, two short, blunt hairs, near the mid-line, arise on a level with the postero-lateral angles. A more pointed hair is situated near each anterior angle and is remote from the mid-line. On the anterior margin are six pairs of bristles. The three lateral pairs are about three times as long as the remainder and are more pointed and straighter and arise ventrally. The other three pairs are blunt and curve downwards and backwards; they arise from the dorsal surface. Ventrally, two oblique, bar-shaped ridges arise about the middle of each half of the labrum. On each of these two very stout, bluntly conical bristles are inserted which point obliquely downwards and forwards.

The antennae (Fig. 10) are one-segmented and greatly reduced and are situated near the outside edge of the mandible on the anterior margin of the frons. They appear as small, thin-walled areas scarcely projecting above the general surface of the capsule. Each bears a bluntly conical, thin-walled appendage which is concentrically striated. In addition there are four to five smaller conical processes.

The mandibles (Figs. 11 and 12) are very chitinous and dark brown in colour, as long as they are broad at the base. They bear two teeth, one above the other, the dorsal one being stouter with a waved inner



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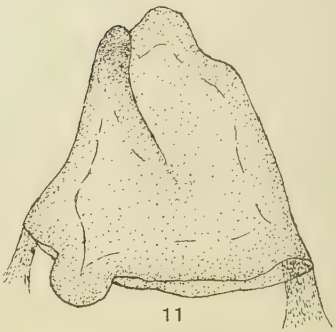

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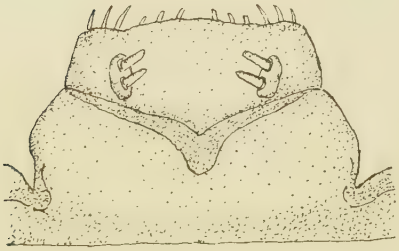

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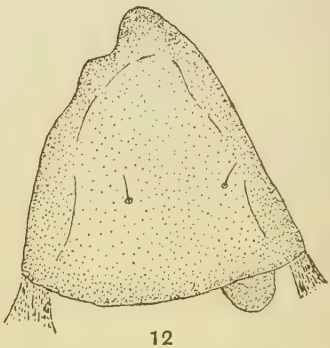

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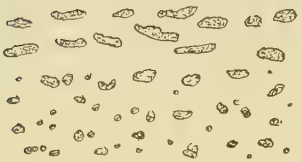

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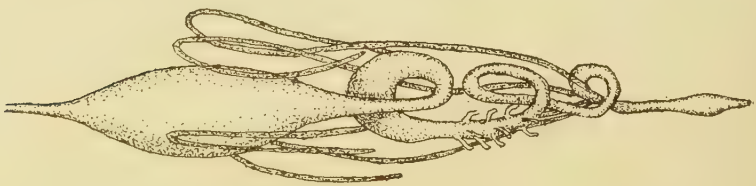

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Fig. 8. Full-grown larva: labrum and clypeus, dorsal view. ×360.
Fig. 9. Full-grown larva: labrum and clypeus, ventral view. ×360.
Fig. 10. Full-grown larva: antenna. ×450.
Fig. 11. Full-grown larva: mandible, ventral aspect. ×360.
Fig. 12. Full-grown larva: mandible, dorsal aspect. ×360.
Fig. 16. Full-grown larva: part of cuticle of dorsal side. ×360.
Fig. 22. Full-grown larva: alimentary canal. ×18.

edge. The ventral tooth is a little shorter than the other with a narrow, somewhat pointed tip which is bent slightly downwards. There is a distinct groove between the two teeth. The upper surface of the mandible is even and nearly horizontal, sloping very slightly towards the inner edge, and bears two hairs, one near the lateral edge, and the other about the middle. The ventral surface slopes towards the middle.

The maxilla (Fig. 13) is comparatively long and narrow and bears a hair about its mid-length. The lobus internus is conical and bears a comb of seven long slender bristles, which increase in size from before backwards, on its inner edge. The maxillary palp is two-jointed, the two joints together forming a short conical projection. The rounded tip of the distal joint bears four to five minute cone-shaped sensorial papillae. Below the proximal joint arise two hairs, one laterally, the other in the middle of the ventral surface.

The labium (Fig. 13) is more or less tongue-shaped, broader at the base than at the top. The edges are convex, somewhat sinuous anteriorly. The forward edge is rounded and consists of softer cuticle than the greater portion of the labium. On the inner side of the anterior part are numerous transverse rows of minute cuticular spines or teeth directed backwards. The labial palps are single-jointed, small, ovoid and about as broad as long, and rounded at the tip on which are situated four to five minute conical processes. The palps are widely distant and arise near the anterior edge of the labium and project a little beyond it. Four pairs of hairs, almost equidistant in position, occupy the length of the ventral surface of the labium and are inserted near the lateral margins.

The thorax. The thoracic segments increase in size from before backwards. They are slightly shorter and are much less arched dorsally and ventrally than the abdominal segments.

The prothorax, which is slightly longer than the other two thoracic segments, bears on the dorsal surface two shields, more or less rectangular in shape, which are separated in the mid-line by a narrow streak of white cuticle. These shields occupy about two-thirds of the length of the segment. Ventrally, three shields are found (Fig. 14). The centre one is irregularly diamond-shaped and stretches over almost the entire length of the segment. On each side of this occurs a narrow elongate shield of irregular outline, stretching over two-thirds of the segment. White linear areas separate the shields. The cuticle of these shields is devoid of spines and is smooth and polished.

The meso- and metathorax have, in the position where the legs would occur, small circular areas of a dark brown colour. Trägårdh

(1910) states that *Rh. quercus* larva has the "cuticular teeth" coalescing to form "a pair of small irregular rings which are probably of some use in locomotion." In *Rh. alni*, however, these patches, while circular in

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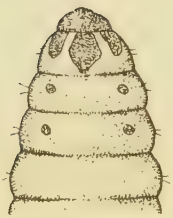

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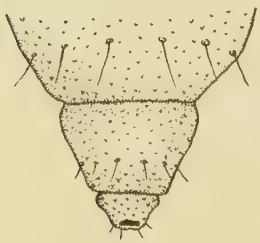

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Fig. 14. Full-grown larva: anterior end, ventral aspect, to show the thoracic shields and markings. ×18.

Fig. 15. Full-grown larva: pygidium, ventral aspect. ×80.

Fig. 19. Pupa: posterior end of abdomen, ventral aspect. ×55.

shape, are not rings and, moreover, are pigmented areas without any special concentration of cuticular teeth.

Von Frauenfeld (1863), as has already been cited, describes the larva of *Orchestes ulmi* De G., but according both to Miller, to whom he showed the adults which had been reared from the larval state, and to Kalten-

bach (1874), he really had been handling *O. alni*. In the description of the larva he says: "Der Kopf ist sehr dunkel und am ersten Ringel steht ein tiefbraunes Nackenschild. Auf der Unterseite dieses Ringels steht ein beträchtlicher dunkler Fleck in der Mitte und beiderseits ein etwas kleinerer. Auf den nächsten zwei Ringeln steht am Rücken jederseits ein feiner dunkler Punkt nicht fern von der Mitte, ebenso auf der Unterseite, nur daselbst ganz an den Rand gerückt."

The two fine dark points on the dorsum of the meso- and metathorax to which von Frauenfeld alludes have never been observed in the numerous larvae examined by the writer.

The abdomen. The abdomen consists of ten distinct segments, the first six of which are almost equal in size, while the remaining four successively diminish in height and width and form a gradually tapering tail. Viewed laterally, segments 1–7 appear to be arched into broad conical projections about one-quarter as high as they are wide at the base. They are less highly arched than appears to be the case in *Rh. fagi*, and *Rh. quercus* larvae. At the top of each arch is a narrow oval area across which a cuticular fold runs; no cuticular teeth are found in this position. The ventral surface of the abdominal segments is gently curved. The small tenth segment, the pygidium (Fig. 15), is conical in shape and about as long as it is broad.

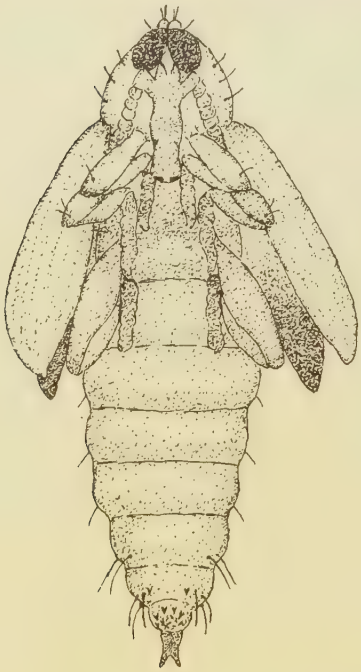
The cuticle. The cuticle (Fig. 16) has numerous small thickenings or pigmented patches which are arranged more or less in transverse fashion and vary in size from mere dots to very small irregular elongate patches. Minute cuticular spines, which are also coloured dark brown, are found on the cuticle, especially on the terminal segments on the abdomen.

THE PUPA.

The pupa (Figs. 17 and 18) is at first white, but gradually darkens to cream and finally becomes light yellowish-brown. It is 4·2 mm. long. The general shape from ventral and dorsal aspects can be seen from the figures. Distinguishing bristles are found on the pupa. The rostrum bears a pair, near its base, close to the eyes, while a second very small pair is situated almost laterally about one-third of the length of the rostrum from the distal end. This arrangement is, therefore, quite different from that described by Trägårdh for *Rh. populi* and *Rh. fagi*. Immediately above the eyes, situated on two low brownish tubercles, closely approximated, are four bristles, two on each tubercle. Immediately lateral of each knob is one bristle which is comparatively short. Ventrally, three pairs of bristles are to be found on the genae. These are situated

so as to form a line which runs laterally. Dorsally, on the vertex are two pairs of bristles which arise close to the mid-line just anterior to the edge of the prothorax.

The pronotum also bears two pairs of bristles near the base of the elytra and lateral in position. On the mesonotum are two pairs of small bristles which form a transverse line about the middle of the segment.



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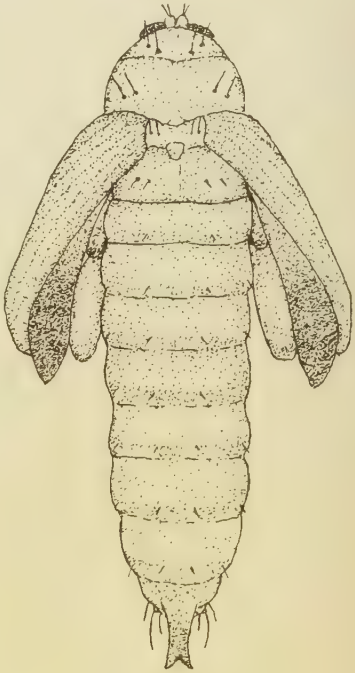

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Fig. 17. Pupa, ventral aspect. ×22.
Fig. 18. Pupa, dorsal aspect. ×22.

Similarly, on the metanotum are two pairs of bristles which, however, are longer and placed more laterally.

The abdomen is seen to consist of eight segments when viewed dorsally, the terminal one projecting into a narrow chitinous appendage, bifid at the tip, each tip bearing a very small claw-like seta. Viewed ventrally (Fig. 19), the abdomen shows the concentration of the last segments which is found in the adult. The ninth and tenth segments are telescoped within the eighth, which bears two small stout chitinous

spines some little distance on each side of the mid-ventral line, and three pairs of long hairs laterally. These hairs arise from slight protuberances of the pupal cuticle. Segment nine is circular in outline and bears six chitinous spines similar to those of the preceding segment. These spines are situated nearly equidistant from each other and arise in the middle of the segment forming three distinct pairs. The tenth segment is very small, appearing almost as a tiny tubercle devoid of any armature.

BIOLOGY.

This insect passes the winter in the adult stage. In August the beetles become less frequent on the elm foliage, and by September have practically all disappeared. During this period they begin to secrete themselves for the autumn and winter under the protection of the old, half-detached bark of elms. This is the principal site for hibernation, but they have also been occasionally found under leaves and plant refuse surrounding the trees. They show no preference for any side of the tree, but frequent more commonly the loose bark of the lower trunk.

Their time of appearance in spring is during April, and after emerging from their winter shelter they begin to feed on the buds and expanding leaves and mating occurs. When the foliage is a little further advanced egg-laying commences. The female selects the mid-vein or one of the larger secondary veins on the under surface of the leaf and, after biting out a suitable cavity with her snout, lays in it a single egg. As a general rule only one egg is deposited in each leaf, although on occasion two or more have been dissected from the mid and secondary veins of a single leaf. Several times it was observed that a number of pieces along the veins had been bitten out, although no egg had been deposited in the cavity, suggesting that the insect had been searching for a suitable spot or a sufficiently large vein in which to oviposit.

The egg hatches in a week or thereabouts during early May, and the newly emerged larva commences feeding activities in the blind end of the cavity in which it lies and proceeds to mine the inner portion of the vein. Comparatively soon, however, it cuts through the vein and attacks the leaf parenchyma, taking care not to rupture either upper or lower epidermis. At first the mine is narrow, thread-like and linear, but with the growth of the larva the mine gradually increases in width and later changes into a blotch-mine of an irregular shape. As the larva proceeds with its feeding, devouring the parenchymatous tissue, the epidermis of the leaf both upper and lower, which still remains intact, gradually dies and assumes a brownish colour; since there is no outlet, masses of

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Fig. 20. Elm leaf showing damage done by *Rh. alni* L. Nat. size. Underside of leaf to show the eaten-out portion with cocoon.

Fig. 21. Upper side of elm leaf to show mined portion. Nat. size.

black granular excrement may be observed within both the linear and blotch parts of the mined area. The larva eats and burrows in all directions so that the mine has no regular shape. Under observation with a binocular microscope it was seen to lie comparatively stationary, slowly twisting the body from one side to the other and devouring the parenchyma in an arc, enlarging the mine by successive narrow streaks, similar to the action in scything grass.

Trägårdh includes in his paper a short discourse on the larvae he studied with regard to their adaptation for mining purposes, the adaptations falling into two groups, viz.: (*a*) for feeding purposes, and (*b*) for locomotion. His remarks on *Rh. fagi* and *Rh. quercus* apply equally well to the species under consideration. Perris (1876) also noticed the adaptation of the larvae of the genus "Orchestes" for mining purposes, and sums up the situation in the following words: "On comprend que des larves qui ont ce genre de vie ne soient pas conformées tout à fait comme celles des fruits, des fleurs et des écorces; elles sont, en effet, droites, plus souples, plus régulières dans leurs formes, moins pourvues de plis et de mamelons latéraux, plus déprimées; leur tête est plus petite et plus aplatie, leur mamelon anal un peu plus allongé."

On an average the larva becomes full grown in three weeks and has by this time mined an appreciable portion of the leaf. It thereupon proceeds to spin a spherical cocoon thus causing an expansion in a part of the upper and lower surfaces of the mine which takes the form of a blister a little less than a quarter of an inch in diameter (Figs. 20 and 21). The cocoon, which is very thin and formed within the leaf itself, is constructed in a similar manner to that of *Rh. fagi* and *Rh. quercus*, the thread used in its formation issuing from the anal aperture of the larva. A dissection shows the alimentary canal of the larva (Fig. 22) to be the same as that of *Rh. fagi* described by Trägårdh and probably the Malpighian tubes serve as the spinning glands, the conical shape of the pygidium being adapted for the purpose of spinning. The cocoon is firm-walled, the threads composing it forming three or four layers, individual threads, in places, having coalesced. That several weevil larvae construct a cocoon in which to pupate is now a well-known fact and the method of production of the thread employed in its formation has been a subject of interesting study. Knab (1915) in discussing the secretions used by Rhyncophorous larvae in cocoon-making mentions that "there is good reason to believe that the substance constituting the cocoons of weevils is at least for the greater part a product of the Malpighian tubes and therefore voided through the anus." Even Perris

in 1876 in summarising the characters of the genus *Orchestes* writes in this connection that "...au dernier moment, la larve s'enveloppe d'un cocon qu'elle confectionne à l'aide de ses mandibules et de ses palpes avec une substance mucilagineuse qui sort par l'anus."

After the completion of the cocoon the larva changes into the pupal stage which averages a week in duration. By about the middle of June the first adults appear, having bitten their way through cocoon and leaf epidermis. As time passes more adults escape and these continue feeding on the elm leaves until August when they proceed to seek out suitable hibernating quarters. So far as was observed in the Warwickshire area, there was only the one generation in the year.

The damage done by the elm leaf beetle is two-fold and consists in the mining of the larvae and the feeding punctures of the adults. Little need be said regarding the mining operations as these have already been mentioned. The adults, however, are destructive and may cut holes through the tender foliage and occasionally destroy it outright. More commonly the beetles feed on the underside generally of leaves which have become full grown; they eat out the soft mesophyll tissue and leave the upper epidermis intact, thus producing numerous shallow pits. As time passes the unconsumed epidermis dies and becomes detached leaving a hole in the leaf. Leaves which have been heavily attacked often appear quite riddled with holes.

The injuries of both adult and larva have a serious effect on the foliage, especially when the insect is present in large numbers, and upon the trees themselves, making them incapable of vigorous growth and even causing the death of the lower small branches. This damage has been observed by several investigators, amongst whom mention may be made of Ritzema-Bos (1887) who stated that *Rh. alni* had appeared in Holland as an important pest of elms, depriving older stems completely of their leaves, while some of the trees, even after two years' defoliation, had died. Bertoloni (1844) also discoursed at length on the deleterious effects of the weevil and pointed out that in Italy the ravages of the insect deprived the cattle of fodder, and impaired the strength of trees to such an extent that they were barely able to remain alive, while the resulting timber bore the distinguishing mark of being rather bad in character, thus causing any remaining good elm wood to be sold at the very highest price. There are numerous other references to damage of a similar character to both elm and alder in the literature concerning this weevil.

PARASITES.

During the course of the investigation it was observed that a considerable number of hymenopterous parasites were attacking the beetle in its larval and pupal stages. On first opening up the blotch-mines parasitic larvae of varying sizes were found lying on the coleopterous larvae or pupae, or close by them, and in such instances the individuals of *Rh. alni* were dead, deformed and more or less shrivelled up. From the beginning of July onwards the beetles became scarcer owing to increased parasitism, and by the third or fourth week of July an examination of elm leaves produced evidence of heavy parasitism to an extent, at a rough estimation, of about 40 per cent. Examination of attacked leaves with a binocular microscope revealed the presence or absence of parasites, and a considerable number of specimens of leaves which showed that the enclosed beetle was being attacked were carefully preserved and the parasites bred from them for identification purposes.

It is of interest to note that Escherich (1923) mentions that Ratzeburg attributes, as being parasites of four species of *Orchestes*, 48 different ichneumons of which the greatest number belonged to the Chalcidoidea. Trägardh (1910) succeeded in obtaining from *Rh. populi* two different species of chalcids whose specific determination is not given; he makes no mention of having discovered any parasites of *Rh. quercus*, but Lyle (1920), in writing on the Sigalphidae, mentions *Sigalphus pallidipes* Nees and *S. caudatus* Nees as having this beetle as a host. From *Rh. fagi* only very few parasites, one of which proved to be an undetermined cecidomyid, were found by Trägardh, but Lyle notes *Tetrastichus ecus* Wlk. as being a primary parasite of this species.

From the material gathered in Warwickshire eight distinct species of parasites have been reared and the writer is greatly indebted to Dr Waterston for having kindly identified them as follows:

Superfamily: Chalcidoidea	
Family: Pteromalidae	
1. *Habrocytus orchestis* Ratz.	Primary parasite
Family: Eulophidae	
2. *Pnigalio cruciatus* Ratz.	? Primary parasite
3. *Chrysocharis orchestis* Ratz.	? Primary parasite
4. *Pleurotropis* sp.	Secondary parasite
5. *Tetrastichus cyclogaster* Ratz. var. *obscurata* Ruschka	Primary parasite

Family: Eulophidae (*continued*)

6. *Tetrastichus* (*geniocerus*) — Secondary parasite
7. *Cirrospilus pictus* Nees — Secondary parasite

Superfamily: Ichneumonoidea

Family: Braconidae

8. *Sigalphus pallidipes* Nees — Primary parasite

Key to the larvae of *Rh. populi*, *Rh. fagi*, *Rh. quercus* and *Rh. alni*.

(Adapted from Trägårdh.)

Trägardh (1910) concludes his paper by a key to the identification of the larvae of the three species which he describes. The present writer has taken the liberty of using this key with minor alterations and additions to include the larva of *Rh. alni*.

1. Body flattened, with lateral intersegmental constrictions; black patches on dorsal and ventral sides; pygidium rounded; one prosternal shield; no cocoon **Rh. populi**

   Body rounded, with dorsal intersegmental constrictions on the first to the seventh abdominal segments; no markings on dorsal surface of body; pygidium conical and pointed; three prosternal shields; cocoon **2**

2. Cuticular spinulae, stigmata and pygidium colourless **Rh. fagi**

   Cuticular spinulae and stigmata dark brown; pygidium variable **3**

3. Pygidium dark brown; meso- and metasternum each with two irregular rings of coalesced cuticular teeth **Rh. quercus**

   Pygidium colourless; meso- and metasternum each with two dark brown patches **Rh. alni**

Acknowledgments.

The writer desires to express his thanks to Dr R. S. MacDougall for his helpful advice and criticism given during the course of the work, and to Dr J. Waterston for having been so kind as to determine the species of the parasites reared from this insect.

The major portion of the work was carried out in Warwick and was completed at the Institute of Agricultural Parasitology.

SUMMARY.

The leaf-mining habits of the species of the genus *Rhynchaenus* (syn. *Orchestes*) are well known and *Rh. alni* L. was found in Warwickshire, England, to be causing a considerable amount of damage to the foliage of elm trees through the mining of the larvae and feeding of the adults. This species is known to attack both *Ulmus campestris* and *Alnus glutinosa*, and a historical review of the literature dealing with the beetle has been written in an attempt to elucidate the misunderstandings and settle the controversy concerning the host plant and specific name of the insect.

Brief descriptions of the adult and egg have been given. The first stage larva, which differs from later stages, has been shortly described, while a note has been added on the intermediate stages. The external morphology of the full-grown larva and of the pupa has been described in detail.

The insect has but one generation in the year, in the Warwickshire area, and the life history and general biology have been discussed.

During the investigations it was observed that a considerable number of hymenopterous parasites were attacking this insect. From material gathered, eight distinct species of parasites have been reared, and identified from *Rh. alni* L. of which seven belong to the Chalcidoidea and one to the Ichneumonoidea.

The paper has been concluded with the addition of a key, adapted from Trägårdh (1910), of four species of the genus which have now been described in detail.

LITERATURE.

BERTOLONI (1844). De duobus insectis Ulmo campestri et Pyro malo infensis. *Nov. Comment. Acad. Scient. Bonn.* T. VI, 459–64.

DE GEER (1775). *Mémoires pour servir à l'histoire des Insectes*, T. V, 260–2.

ESCHERICH (1923). *Die Forstinsekten Mitteleuropas.* Paul Parey, Berlin. Zweiter Band, 340, 416 and 418.

FOWLER (1891). *The Coleoptera of the British Islands.* Reeve and Co., London. Vol. V, 254–9.

FRAUENFELD, G. R. VON (1863). Beitrag zur Metamorphosengeschichte aus dem Jahre 1862. II Die Rüsselkäfer; III Verschiedene Metamorphosen. *Verhandl. der zool.-bot. Gesell. in Wien*, XIII, 1225–6 and 1235–6.

FROST, S. W. (1925). The Leaf-mining Habit in the Coleoptera. *Ann. Entom. Soc. America*, XVII, no. 4, 457–67.

HERBST (1795). *Naturg. all. bek. Insekt.* T. VI, 426.

HOUSER, J. S. (1923). The Apple Flea-weevil, *Orchestes pallicornis* Say. *Bull. Ohio Agric. Exper. Stat.* June, no. 372.

Kaltenbach (1874). *Die Pflanzenfeinde aus der Klasse der Insekten.* Hofmann, Stuttgart. 536.

Kleine, R. (1924). Über die Larvenminen einiger Orchestini. *Zeitschr. f. wissen. Insektenbiol.* xix, 203–207 and 251–4.

—— (1925). *Ibid.* xx, 24–7 and 44–53.

Knab, F. (1915). The Secretions employed by Rhyncophorous Larvae in Cocoon-making. *Proc. Entom. Soc. Washington*, xvii, 154–8.

Linnaeus (1758). *Systema Naturae.* 10th ed. 381.

—— (1767). *Ibid.* 13th ed. Vindobonae. Lib. i, pars 2, 611.

Lyle, G. T. (1920). Contributions to our Knowledge of the British Braconidae—No. 5, Sigalphidae. *Entomologist*, liii, no. 682, March, 56–60.

Nüsslin, O. (1922). *Forstinsektenkunde.* Paul Parey, Berlin. Dritter Teil, 240.

Perris, E. (1876). Larves des Coléoptères. *Ann. Soc. Linn. Lyon*, xxiii, 402.

Reitter, E. (1906). *Catalogus Coleopterorum Europae.* 2nd ed. 687.

—— (1916). *Fauna Germanica—Die Käfer des deutschen Reiches.* K. G. Lutz' Verlag, Stuttgart, v, 220–1.

Ritzema-Bos (1887). Beschädigungen von Ulmen durch den Rüsselspring Käfer, *O. alni. Die Landw. Versuchs.-Stationen*, xxxiv, 116–97.

Rupertsberger, M. (1880). *Biologie der Käfer Europas.* Luiz a. d. Donau. 213.

Stephens, J. F. (1839). *A Manual of British Coleoptera or Beetles.* London. 230.

Trägårdh, I. (1910). Contributions towards the Metamorphosis and Biology of *Orchestes populi*, *O. fagi* and *O. quercus*. *Arkiv för Zoologi*, Stockholm, vi, no. 7.

Victorian History of the Counties of England, The.—Warwickshire—1904. Ed. H. A. Doubleday and Wm. Page. Arch. Constable and Co., Ltd., London. Vol. i, 121.

Xambeau (1915). Mœurs et Métamorphoses des Insectes. *L'Échange, Rev. Linn., Moulins*, xxxi, nos. 368 and 369, Aug. and Sept. 33–40.

(*Received February 14th*, 1928.)

OBITUARY NOTICE

Professor W. JOHANNSEN

(Honorary Member of the Association of Economic Biologists.)

(With Plate XXXIX.)[1]

Wilhelm Ludvig Johannsen, a son of Colonel O. Johannsen, was born in Copenhagen on February 3rd, 1857. The family later moved to Helsingoer where Johannsen passed through the secondary school and at the age of sixteen became a pharmaceutical apprentice at the local drug store. His evenings and spare time were given to study and, although he never became a university student, he obtained his pharmaceutical degree in 1880 and, in the same year, was appointed Assistant to Kjeldahl in the chemical department of the Carlsberg Laboratory. There he worked for seven years, and it was during this period that his interests in botany crystallised out. During 1883–4 he was able to spend some time studying plant physiology in the laboratory of Pfeffer at Tübingen and the stimulus of this master led Johannsen to make plant physiology his own. The quality of his work was recognised by his appointment in 1892 to a Lectureship in Plant Physiology at the Royal Agricultural and Veterinary College of Copenhagen where, eleven years later, he was promoted to a full Professorship. In 1905 he was called to the Chair of Plant Physiology in the University of Copenhagen where he remained until his death on November 11th, 1927. Johannsen was married at the age of thirty-two, widowed in 1919 and leaves four children.

Johannsen was no traveller, but in 1911–12 he visited the United States of America where he had been invited to lecture at several universities. His person was well known in the northern European countries and his last visit to England was on the occasion of the British Association meeting at Hull when he was an honoured guest. In his later years he received wide recognition and many distinctions were conferred upon him. In 1910 he received the treasured degree of Doctor Medicinae, *honoris causa*, from his adopted University of Copenhagen, and in succeeding years honorary degrees were conferred by the Universities of Freiburg, Gröningen and Lund. He became an Honorary Member of many foreign scientific societies and in 1923 was elected to Honorary Membership of the Association of Economic Biologists. The honour dearest perhaps of all, and one he barely lived to receive, was the dedication to him on his seventieth birthday of a special volume of *Hereditas* by his colleagues and fellow-workers of the Scandinavian Mendel Society.

Johannsen was no laboratory hermit and his active temperament, many-sided knowledge and wide interests found scope outside his personal scientific discipline. For twenty-two years he served as Treasurer of the Royal Danish Society of Sciences; he was an active member of the Commission of Economic Administration of the

[1] I am indebted to Dr med. Eric W. Johannsen, son of Professor Johannsen, for the photograph of his father which was taken some eighteen months before his death.

University of Copenhagen and his administrative abilities, his tact and wise counsel were supremely recognised by his election as Rektor Magnificus of the University during the troubled period 1917–18. He served for several years as Chairman of the Danish Commission of Seed Control and his wider interests were shown in his work as a member of the Danish Alcohol Commission and the active part he played in many other important public services.

During his period at the Carlsberg Laboratory Johannsen's main scientific work lay in the study of the physiological processes occurring in ripening barley seeds, and his researches made a very solid contribution to knowledge in this field. This work was mostly published in the Reports of the Carlsberg Laboratory and the *Botanische Zeitung*, but his important studies carried out in Pfeffer's laboratory dealing with the influence of various oxygen concentrations upon respiration in seedlings appear in the rare first volume of the collected works of the Botanical Institute of Tübingen.

After his appointment to the Agricultural College of Copenhagen Johannsen continued his barley investigations and in the Carlsberg Report for 1898 published a paper "On variability with special reference to the ratio between weight of grain and percentage of nitrogen in barley," which showed that his mind had already been interesting itself in directions where later he became a master guide. His new post gave him however a more general scope and he entered fully into the problems of the wider field of plant physiology which were then attracting the attention of investigators. His keen interest in the applied aspects of botany led him to choose economic plants as his subjects and to attack problems of practical importance. A favourite plant was the Lilac, and mention may be made of two characteristic researches that were carried out with this plant. One, published in collaboration with E. Warming in 1901, showed the absence of colour in blue lilac when grown above the optimum temperature for the formation of anthocyanin and indicates the way in which he was already feeling towards the distinction which belongs more to him than to any other of "phenotype" and "genotype." Indeed, the little word "gene" was first coined and used by Johannsen. The second research, one that brought him perhaps his greatest popular recognition, was the account he published in 1906 of an ether treatment he had discovered for forcing into premature flower the cut winter shoots of lilac and other plants. This treatment which was immediately and successfully adopted into horticultural practice was the forerunner of all modern methods of forcing. The quality and practical value of this work caused the Royal Horticultural Society in 1906 to bestow upon Johannsen its Veitch Medal of Honour.

It was towards the middle of his period at the Agricultural College that Johannsen commenced the researches for which he became best known and which have given him his enduring place in the history of biology. These were the famous experiments with beans which led to the enunciation of the principle of the "pure-line." Fortunately his paper "Über Erblichkeit in Populationen und in reinen Linien," published in 1903, appeared after the rediscovery of Mendel's studies, when biological interest was keenly alive to the possibilities of genetic research, and its epoch making character was immediately recognised. The work went to the very root of conceptions of genetic purity, it introduced exactness into the study of variability and laid the simple foundation upon which all methods of experimental genetic analysis have been built. With the passing of time its theoretical and pragmatic values have been increasingly recognised and, although it is conceivable that genetic science would have progressed

in the absence of the concept of the "pure-line," one can only think of it as stumbling and blind, a child that had never learned to walk.

From this time onwards Johannsen became more and more attached to the study of genetics and in 1905 published the Danish edition of his *Elemente der Exakten Erblichkeitslehre*, a small volume containing fifteen lectures. Four years later an enlarged German edition was issued and was at once received as an authoritative work of outstanding importance. In his preface, Johannsen stated that "Das Prinzip der 'reinen Linien' is hier mit dem 'Mendelismus' in Verbindung gebracht; näher betrachtet sind Reinkultur und Kreuzung (eben der reinen Formen) gleich wichtige analytische Mittel der Erblichkeitsforschung, die einander ergänzen. 'Mendelismus' und 'reine Linien' haben auch in der schönsten Weise ihre Resultate gegenseitig bekräftigt und dadurch die Lehre von der Selektion in richtigeres Licht gestellt." Enlarged editions of the volume were demanded in 1913 and in 1926, the last edition containing thirty lectures and over seven hundred pages. The book as it stands to-day is a treasury of learning and critical judgment; the work of a great man. The book has never been translated into English and the influence it has had on genetic methodology and perspective has not been perhaps fully recognised by English-speaking biologists. It were a fitting monument to Johannsen that an English translation of his book should be made.

During the last two decades of his life Johannsen contributed extensively to scientific journals and encyclopaedic "Handbücher." One of his most noteworthy smaller papers appeared in the *American Naturalist* at the time of his visit to the United States in 1911, and contained a statement, model in clearness and brevity, of his "Genotype Conception of Heredity." In later writings ("On nogle Mutationer i rene Linier," *Biol. Arb. til E. Warming*, 1911, "Experimentelle Grundlagen der Deszendenzlehre," *Kultur der Gegenwart*, 1915, and especially "Some remarks about units in heredity," *Hereditas*, 1923) he returned to this subject. His searching, questioning mind is perhaps best shown in his last paper from which the following extracts are taken:

"But, however far we may proceed in analysing the genotypes into separable genes or factors, it must always be borne in mind, that the characters of the organisms—their phenotypical features—are the reaction of the genotype *in toto*. The Mendelian units as such, taken *per se* are powerless."

"To my mind the main question in regard to these units is this: Are the experimentally demonstrated units anything more than expressions for local deviations from the original ('normal') constitutional state in the chromosome?"

"Is the whole of Mendelism perhaps nothing but an establishment of very many chromosomical irregularities, disturbances or diseases of enormously practical and theoretical importance but without deeper value for an understanding of the 'normal' constitution of natural biotypes? The Problem of Species, Evolution, does not seem to be approached seriously through Mendelism nor through the related modern experiences in mutations."

"*Chromosomes* are doubtless vehicles for 'Mendelian inheritance,' but *Cytoplasm* has its importance too. I cannot here enter into this problem from which in the near future we shall certainly have important views."

Johannsen was always much interested in the development of scientific thought and wrote several papers dealing with such subjects as "Die Vererbungslehre bei

Aristoteles und Hippocrates" (*Die Naturwissenschaften*, 1917), "Hundert Jahre Vererbungsforschung" (*Verhandl. Gesell. deutsch. Naturf. und Aerzte*, 1922) and other aspects of the history of biology. A popular work in Danish entitled, *Heredity from historical and experimental points of view*, first published in 1917, was very successful and quickly ran into several editions.

Johannsen's physiological and genetic studies did not exhaust his botanical energies and he found time to take a keen interest in forestry. In his paper "On the study of heredity with regard to forestry" (*Tidsskr. f. Skovvaesen*, 1909) he foreshadowed many developments which Danish sylviculture has since put into practice.

As a professional botanist Johannsen did magnificent work in his chosen fields, but his larger vision gave him to see that no science can live and grow which exists for its Professors alone. He had the gift of thinking simply, the art of writing in the vulgar tongue so that all who wished might understand, and he had the will to give his knowledge to the people. In consequence his popular books were immensely successful, especially *Biologi*, published in 1921, and *Arveligheđ*, of which a fourth edition appeared in 1923. His influence on the teaching of biology in Denmark and on the attitude of the Danish people towards the study of nature can hardly be overestimated. He came to the people, not as a Professor to lecture, but as one of themselves, a friend and beloved teacher of whom children and grown men and women delighted to learn.

Johannsen's outlook on human society and life, the way in which he saw man, was not dominated and clouded by his genetic studies. His vision, like that of his fellow master Bateson, ranged more widely and he saw the cultural as well as the biological setting of the human panorama. His sane and balanced views are well shown in the last chapter of his *Elemente* (1926) where he writes:

"Zur Euthenik gehören alle sozialen Besserungen der persönlichen Lebenslagen, Erziehungsveranstaltungen, öffentliche Gesundheitspflege u. dgl. mehr; zur Eugenik im engeren Sinne gehören die Bestrebungen züchterischer Art in den Populationen; Bestrebungen, die äusserst schwierig zu praktizieren bzw. zu regulieren sind. Euthenik und Eugenik müssen einander stützen—nicht gegenseitig verketzern; im einzelnen ist es auch nicht leicht zu entscheiden, wo Euthenik in Eugenik übergeht." And he concludes:

"Für die grosse Masse der Mittelmässigkeiten mag Erziehung von entscheidender Bedeutung im Leben sein; darin liegt die eminente Wichtigkeit der Erziehung im allgemeinen—Ausnahmebegabungen werden sich meistens auch ohne spezielle Erziehung manifestieren. Dabei aber kann man nicht umhin, in Erziehung und Schulung überhaupt, Faktoren zu sehen, die an und für, sich gegen Originalität feindlich sind. Es geht aber hier wie mit Feuer und Wind; der Wind löscht das Flämmchen; stärkt aber das kräftigere Feuer. Übrigens ist Originalität an sich durchaus nicht immer des guten!"

The keen and crystal intelligence, the imaginative insight and the utterly cool and sober brain which Johannsen the scientist devoted to the quantitative analysis of physiological and genetic problems were balanced in Johannsen the man by purely human qualities no less great; a warm heart sweet with the milk of human kindness, rare sympathy and a noble gift of understanding. His world was "very full of a number of things," and he drank deeply of life for his interests were quiveringly alive and widely embracing. He had had no university education, but where he lacked

JOHANNSEN, Professor W.—Obituary Notice (pp. 699–703).

teachers he taught himself. His mother tongue, English, French and German he spoke fluently and he was familiar with Latin and Greek. He was an expert mathematician, deeply learned in philosophy and aesthetics and more widely read in the world's literature than most men. An unusual memory and a sensitive appreciation of life's happenings, not least the humorous ones, made his talk delightful and his companionship a thing of joy. Young scientists coming under the influence of his rich and generous personality found in him a tower of inspiration and a fount of wise counsel. In his lifetime Johannsen reached goals such as few attain and saw himself esteemed as he had deserved. But underneath and unhidden in Johannsen the Rektor Magnificus, the Scientist whom the world delighted to honour was the genuine and pure humanity of Johannsen the man, a wise little figure brimming over with good humour and kindliness; a man loved as it has fallen to the lot of few to be loved by their fellow men.

WILLIAM B. BRIERLEY

REVIEWS

"The Biological Control of Prickly Pear in Australia." By A. P. Dodd. *Council for Sci. and Indus. Res. Bull.* 34. Melbourne. 1927. Pp. 44; 9 plates.

The area of Australia infested by prickly pear (*Opuntia* spp.) is estimated to amount to 60,000,000 acres in Queensland and New South Wales, and, a few years ago, it was stated to be spreading at the rate of a million acres a year, but this increase is no longer being maintained. In the main the affected areas embrace natural grazing country, where the land is worth less than £3 per acre. Under such conditions chemical or mechanical control is impracticable except in lightly infested country, where such methods can be economically carried out. Prickly pear is unlikely to become a pest in good agricultural land, because the value of the latter permits of the destruction of this plant at a relatively economic cost. In their native terrain in North and South America about 350 species of *Opuntia* are known, but none is a very serious enemy, yet of the few kinds introduced into Australia, at least four are to be regarded as major or minor pests. In America insects, diseases, and other agencies keep the prickly pear within reasonable bounds, whereas in Australia such natural controlling factors are wanting and there is little to check the reproduction and spread of the pest. The Prickly Pear Board of Australia is concerned with an attempt to bring about a condition of biological equilibrium by the introduction of insects and plant diseases likely to act as natural checks. Eight years have elapsed since the Board's formation and the position of the problem of prickly pear control up to the end of May, 1927, is described in *Bull.* 34 of the Council for Scientific and Industrial Research of Australia. Its author, Mr A. P. Dodd, describes the scheme from its inception and discusses the various insects that have been or are being introduced from the New World. The biological control aimed at depends upon the introduction of a complex of organisms working together in destructive unison, and he tells us that the investigations will not be complete until every prickly pear area of any extent has been explored for insects or disease organisms likely to be of service. It needs to be recollected that the two chief pest pears in Australia are *Opuntia inermis* and *O. stricta*, while several others are of minor importance. This fact complicates the problem for the reason that a particular species of insect may prove effective against one kind of prickly pear, and yet be of little value with respect to other kinds of those plants. Officers of the Board have been and are still engaged in studying the insects affecting *Opuntias* in their native surroundings. They have covered widespread Cactus areas in North America, where field stations have been set up, and have also visited South America and the West Indies. In work of this character it is important to study on the spot, not only the insects actually attacking prickly pear, but also the natural parasites of such insects. The exclusion of the parasitic forms from Australia is of prime importance if their host insects are to multiply freely and vigorously attack the prickly pear. At the Board's Station at Urvalde in Texas extensive biological work is being prosecuted, and all the most promising Cactus-feeding insects are being bred under caged conditions: furthermore, their life-histories are being worked out, such insects are being tested relative to the possibilities of their attacking economic plants and freedom from parasites is being ensured. This lengthy groundwork is a necessary preliminary before any species of insects can be safely shipped to Australia. The material received from America is transferred to quarantine buildings at Sherwood near Brisbane, where they are bred through one or more generations as an additional safeguard against the accidental introduction of their parasites. At Sherwood, also, further tests are conducted with respect to the possibility of the introduced insects attacking crops and

other useful plants. From Sherwood the insects are eventually forwarded to acclimatising and breeding centres where, as a rule, the first liberations are also carried out. The progress of the liberated insects in the vicinity of a field station can be closely watched and its destructive effects in the field noted. The acclimatisation of North American insects in a country where they are faced with opposite seasonal conditions naturally presents considerable difficulties. Generally it has been found that repeated shipments of a species of over a period of one or more years have been necessary before it has become established, but in a few cases efforts have failed altogether. Once the preliminary liberations have been effected and an insect has established itself locally, its distribution over wide areas of country has then to be provided for. The Board itself is not in a position financially to undertake general distribution and the latter becomes a matter for the States concerned, acting in conjunction with the Board.

Among the various insects peculiar to the Cactus family there exists a variety of internal-feeding or boring larvae of various moths. Of the various species already liberated the most promising is *Cactoblastis cactorum* which is now firmly established in Australia, where over nine million eggs have been distributed and liberated in a little over a year. It is regarded as the most destructive insect yet introduced and has already destroyed much of the pear growth in several localities. It is especially partial to *Opuntia inermis* but also attacks *O. stricta*, and so far is the only important enemy that will attack *O. aurantiaca*. Several species of *Melitara* have been introduced: the solitary species *M. junctolincella* is now firmly established in several localities but less stress is now laid upon it owing to the greater potentialities of the *Cactoblastis*. Among the social species of *Melitara* no very marked success has yet been achieved, but it is hoped that one or more species will become firmly established in the future. Of the cactus bugs of the genus *Chelinidea*, four forms were introduced in 1921, and all were readily acclimatised: the species *C. tabulata* from Mexico has proved most suited to Australian conditions and is now well established in many localities in Queensland and New South Wales, even to abounding in millions. The fact that it multiplies rapidly, attacking the young growth of prickly pear and the fruits, renders this species a promising adjunct in the work of biological control. The introduction and spread of cochineal insects have presented few difficulties and there are now very few areas of prickly pear free from these insects. The Indian cochineal, *Dactylopius indicus*, introduced from Ceylon in 1913, only attacks *Opuntia monocantha* which, however, is not a real pest in Australia. So effective has been the destructive work of this species of cochineal that *O. monocantha* is regarded as a very rare plant to-day. The wild cochineal *Dactylopius tomentosus* was imported in three strains or races, the Chico strain being most destructive to *Opuntia inermis* and the Texan strain being partial to *O. stricta*, while all three strains attack the tree pear *O. tomentosa* about equally. The best results with cochineal have been obtained with pear growing in dense scrubs, the impenetrable vegetable barrier being gradually broken down and destroyed by its agency. As an offset to the good work of cochineal it must be borne in mind that it is subject to attack by the Australian ladybird *Cryptolaemus montrouzieri* which is now found wherever the *Dactylopius* occurs. The ultimate effects of this predator cannot yet be foretold, but, up to the present, it does not appear to have exercised more than a slight restraining influence. The red spider (*Tetranychus opuntiae*) is native to Texas and, since its introduction into Australia in 1924, it has spread rapidly and quickly reduces many acres of prickly pear to a state of partial collapse. It is more rapid in its attacks than cochineal, but they are more spasmodic and less continuous. The two insects together form a harmonius combine by whose agency there is every reason to anticipate that dense scrub areas of *O. inermis* will be eventually eradicated. In order to illustrate the effectiveness of the red spider a 600-acre area of scrub is mentioned as being four or five feet high in 1924, but to-day 75 per cent. of it has been destroyed. The residue averages only two feet high and grass has sprung up where it was totally excluded previously.

Space precludes the mention of other kinds of insects whose introduction has so far not proved successful or is still being proceeded with. Work on the introduction of bacterial and fungal diseases of prickly pear is as yet in the preliminary stage, and

much fundamental investigation remains to be carried out before the introduction of any of the pathogenic agents concerned can be contemplated. It is possible that certain organisms of this character may prove useful aids in conjunction with insect attacks, though it is not anticipated that any wholesale eradication of prickly pear is likely to result from disease alone.

We can congratulate the Prickly Pear Board and its officers on the extremely encouraging results so far achieved and on the cautious and thoroughly scientific manner in which their work on biological control of prickly pear is being carried out.

A. D. IMMS

Statistical Methods for Research Workers. By R. A. Fisher. Edinburgh: Oliver and Boyd. 1928. Pp. x + 266; 65 + 6 Tables; 12 Figs. 2nd ed., revised and enlarged. 15*s*. net.

It may be stated as a truism that the advance of science depends for its impetus on the elaboration or application of new and more adequate techniques. Of no science can this be said more truly than of Biology which is enlarging its scope continually by the intensive application of techniques borrowed from cognate or even distantly related sciences. Fruitful as this tendency has been, it is not carried through without arousing anxiety among certain workers in the biological domain who look with apprehension on the application of technique borrowed from a science with which they are not concerned, and of which their acquaintance is limited to a familiarity with general principles. This disquietude is grounded, without doubt, in the conviction that, however complicated the technique employed the value of experimental results depends largely on extraneous factors with which the technical method has no concern, in fact that the designing of experiments and anticipation of possible disturbing elements is at least as important as the technique actually employed. This question of design and adequate control differentiates biology from any other of the experimental sciences, and raises problems for the biological worker with which the chemist or physicist is untroubled. The experimental biologist thus needs firstly to design experiments so that maximum "control" is secured, and secondly to design them in such a way that the data obtained may be used with the greatest economy in testing the validity of the hypothesis under trial. A concrete example may be cited in "Field Experimentation," which dates back to the beginning of experimental biology. The problem of "control" in field trials has been a continual source of anxiety to the experimenters in the past, and the method of laying out such trials to secure maximum information with minimum replication, *i.e.* with greatest economy, was not understood. This problem has now been completely solved, and the principles laid bare, by the Statistical Researches of Dr R. A. Fisher. It is not an exaggeration to say that Statistics itself has been almost transfigured by the necessity of meeting the requirements of experimental science. Here then is a technique for Research Workers whose importance cannot be overestimated, and it is the aim of the book under review to supply to biologists "the means of applying statistical tests accurately." That biologists have not been slow in availing themselves of this advantage is shown by the necessity of a new edition of Dr Fisher's work after so short a time. It cannot be said that this book is easy reading, on the contrary, it demands careful and close study from the reader. It will, however, be clear even to a casual reader that the general principles involved in statistical tests of significance are continually being emphasised, and the formal analogy of all the tests used is clearly brought out. A certain perspicacity is required of the reader in distinguishing for himself the principles involved from the subtleties of the arithmetical methods used in the practical working of any one of the tests. These arithmetical methods must clearly be mastered before the tests can be made expeditiously, and those who have used these ingenious labour-saving devices will best appreciate the benefit that Dr Fisher has conferred on them. The second edition follows closely the lines of the first. Some new features have however been added: first a new arithmetical method of fitting polynomial values which ought

greatly to abbreviate the work involved in the fitting of complex regression lines, and, secondly, an extra table giving the values of the 1 per cent. point in the distribution of Z, for which only the 5 per cent. values had previously been published. An extra final chapter has been added bringing together those principles of statistical estimation of which the tests of significance dealt with in the preceding chapters are individual examples. For those who master the implications of this last chapter the general principles involved in statistical estimations of significance will become sufficiently clear to prevent them from making gross misapplication to particular problems (which many biologists would seem to fear), and this in spite of the fact that mathematical proof of the general theorems is not included in this work. Such as require this enlightenment are referred to the original papers in the bibliography. Let it however be realised that biologists continually use techniques borrowed from other sciences without feeling impelled to acquaint themselves with the full content of these sciences, and that in relation to statistical methods their temerity should fail, reflects perhaps the disinclination felt by those biologists with metaphysical leanings to having their science based on a quantitative rather than abstract basis.

F. C. GREGORY

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